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PLASMA CARNITINE, CLINICAL PARAMETERS, AND QUALITY OF LIFE IN PATIENTS RECEIVING HEMODIALYSIS

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PLASMA CARNITINE, CLINICAL PARAMETERS, AND QUALITY OF LIFE IN PATIENTS RECEIVING HEMODIALYSIS.

By

Alison Leah Steiber

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ABSTRACT

PLASMA CARNITINE, CLINICAL PARAMETERS, AND QUALITY OF LIFE IN PATIENTS RECEIVING HEMODIALYSIS.

By

Alison Leah Steiber

Carnitine metabolism and the therapeutic use of carnitine has been a major area of interest in dialysis patients. The purpose of this study was to determine risk factors for suboptimal plasma carnitine concentrations and associated factors in hemodialysis patients and to determine if a defined group of chronic kidney disease (CKD), patients at increased risk for altered carnitine metabolism would respond to L-carnitine with regards to clinical parameters, perceived quality of life, and plasma acylcarnitine moieties. To examine these issues this study was done in two phases. Phase I was a cross sectional, observational study and phase II was a randomized, double-blind, clinical trial, both were conduct at a Midwest dialysis center on patients receiving hemodialysis (HD). Phase I resulted in subjects (n=49) who were 60±16 (mean±SD) years of age and 48% male. 15% had type 1 Diabetes Mellitus (DM), 29% had type 2 DM, and 25% had left ventricular hypertrophy (LVH). The plasma free and total carnitine (FC and TC), acylcarnitine (AC), and acyl-to-free concentrations (A/F) were: 40.3±11.8μm/L, 22.8±7.3μm/L, 17.5±5.9μm/L, and 0.80±0.27, respectively. Blood urea nitrogen (BUN), parathyroid hormone (PTH), and ejection fraction were positively correlated and age and left atrial dilation were negatively correlated with TC. BUN and hematocrit were positively correlated and age was negatively correlated with FC. Subjects who used mannitol or were male had higher concentrations of both FC and TC, respectively. Patients in phase II

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met the following criteria: age >18 years, on HD 3x/wk >1 year, a plasma FC concentration of <40μmol/L and presence of 2 of the following risk factors: > 65 yr, 2 yr duration of dialysis, female, use of aspirin and/or mannitol, have type 2 DM, left atrial dilation and/or LVH. In phase II, 50 patients were randomized into treatment and placebo groups. Patients were blindly treated with 2 g IV carnitine or placebo. The treatment period was for 24 weeks with data collected at 0, 12 and 24 weeks. Of the 50 recruited, 7 died, 7 withdrew, 2 had FC>40μmol/L, and 7 did not complete the final SF36 tool (available data used). Of the remaining 34 patients, the mean age was 68±14 yrs, 38% were female, 45% had diabetes. Mean Subjective Global Assessment, Body Mass Index, energy intake and protein intake were 6± 0.8; 28±7; 17kcals/kg and 0.7g/kg respectively. Mean plasma TC, FC, Short Chain AC, Long Chain AC and A/F carnitine ratio were 36.05+10.64, 18.9+6.5, 11.12+4.01, 6.03+1.91 (μmol/L) and 0.97+0.34. Paired t-test analysis showed significant improvements in the treatment group for role-functioning (21.2±24.7 to 53.9±72), bodily pain (53.5±35.8 to 72.2±31.4), role-emotional (58.9±43.4 to 84.7±29.18), and the SF36 physical (36.1±9.6 to 39.7±8.5, p=0.078) composite score; but no significant changes in the control group. Erythropoietin dose changes were significantly different between treatment and placebo groups from 0 to 24 weeks (-1316±2795 versus 1464±2770 units). All acylcarnitine moieties were significantly increased at 12 and 24 weeks for patients in the treatment, but not control, group except for 2-methylbutynylcarnitine. In summary after 24 weeks of IV carnitine therapy, acylcarnitine moieties increased, SF36 scores improved and erythropoietin doses were reduced.

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This dissertation and all the work involved in its creation are dedicated in loving memory to David Ainsworth and Nancy Ainsworth-Vaughn. They inspired me to become the person I am today. This work was accomplished due to the love, support, and encouragement of so many. Tremendous amounts of thanks must go to the physicians, dietitians, nurses and especially the patients of the Dialysis Center of Lincoln. They voluntarily participated in my research making the impossible, possible. Extra special thanks to Dr. Leslie Spry for believing in this project and to Jen Strong for keeping me organized.

Another big thank you to my lab partners, they made me laugh, laughed at me, and again made the whole experience sane when it should have been insanity. Special thanks to Mia and Abby for traveling with me. Our laboratory and the special environment that it achieved could not have been possible without the leadership and caring of Dr. Weatherspoon. She led us with respect, humor, and love and for that I am grateful. And a huge thank you to Dr. Davis, without whom I would have not data.

Finally and eternally, I thank my family. My mother has been my best critic and best support for as long as I can remember, this endeavor has been no exception. My children have endured many hours in labs (especially on grant days), much time with Dad or Grandma while I was away at meets or studying for tests and occasionally a tired and cranky Mom. I thank them and hope they will forgive me. To my husband, I do not think there are words to express my undying gratitude for all he has done. He is the love of my

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life and my soul mate. This dissertation would not have been accomplished without his presence in my life.

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Acronyms

1. Chronic Kidney Disease	CKD
2. Kilocalorie	kcal
3. Glomerular Filtration Rate	GFR
4. Sodium-dependent organic cation transporter	OCTN2
5. Subjective Global Assessment	SGA
6. Medical Outcomes Short Form-36	SF36
7. Dialysis Center of Lincoln	DCL
8. University committee on Research Involving Human Subjects	UCRIHS
9. Mid-Arm Muscle Circumference	MAMC
10. Body Mass Index	BMI
11. Normalized Protein Catabolic Rate	nPCR
12. Standard Deviation	SD
13. Diabetes Mellitus	DM
14. Left Ventricular Hypertrophy	LVH
15. Left Arterial Dilation	LAD
16. Blood Urea Nitrogen	BUN
17. Hemodialysis	HD
18. Coenzyme-A	Co-A

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Chapter 1

Introduction

The prevalence of chronic kidney disease (CKD) has been on the rise in the United States, at an average rate of 8% a year over the past five years. As the disease progresses from early to advanced stages (Table 1.1), it eventually becomes necessary for dialysis treatment to be initiated. There are currently more than 250,000 individuals in the United States with CKD, undergoing maintenance hemodialysis or chronic peritoneal dialysis. The number of maintenance dialysis patients will surpass a half million by the year 2010. As prevalence of CKD increases so does the cost. In 1993 and 1999, dialysis provider reimbursement by Medicare was 7.12 billion and 10.77 billion dollars, respectively. As the average age in the United States increases so does the mean age of patients on dialysis. In 2001, patients 55 to 60 years old with chronic renal failure who were receiving dialysis had an average life expectancy after initiation of treatment of 4.5 years; the average age for patients on dialysis was 56 years old (1).

Patients with CKD experience lower quality of life, greater morbidity, higher hospitalization rates, and increased mortality as compared with the general population. Characterized by uremia this disease is also associated with clinical complications such as anemia (2), malnutrition (3), osteoporosis (4), tissue and vessel calcification (4), hypertension (5), left ventricular hypertrophy (6), hypertriglyceridemia (7), and fluid retention (8). A number of metabolic functions are affected as kidney failure progresses abnormal concentrations of substances such as urea begin to accumulate in the blood.

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Table 1.1

Stages of Chronic Kidney Disease

Stage	Description	GFR, mL/min/1.73m^2
-	At increased risk	≥60*
1	Kidney damage with normal or ↑ GFR**	≥90
2	Kidney damage with mild ↓ GFR	60-89
3	Moderately ↓ GFR	30-59
4	Severely ↓ GFR	15-29
5	Kidney Failure	<15 (or dialysis)

*with chronic kidney disease risk factors (>60 years of age, hypertension, previous kidney damage, belonging to a ethnic or racial minority.
** GFR = glomerular filtration rate
*** Eknoyan G, Latos DL, Lindberg J. 2003 (9)

Poor appetite, nausea, and restrictive dietary regimes are associated with compromised protein and energy intakes and varying degrees of malnutrition (10). A major well noted secondary effect of renal failure is decreased hydroxylation of vitamin D, which affects calcium metabolism and subsequent uptake into the bones thereby causing osteoporosis. Finally, cardiovascular complications associated with the combination of hypertension, fluid retention, left ventricular hypertrophy, and hypertriglyceridemia may act exacerbate morbidity and mortality in the CKD population (6;7). Depending on the severity and number of these clinical conditions, functional and mental status may be impaired to the point that quality of life is compromised.

Dietary modification is a cornerstone of treatment for CKD patients because of the metabolic alterations, which occur primarily in protein and electrolyte metabolism. In the

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early stages of CKD, protein and electrolytes such as potassium, phosphorus, and sodium are restricted in an effort to preserve kidney function. When patients reach stage 5 CKD and receive dialysis, the dietary recommendations change due to protein and small water-soluble molecules, such as carnitine, being lost in the dialysis process. Protein requirements are elevated. However, there are currently no dietary recommendations for carnitine.

Recently, carnitine (3-hydroxy-4-N-trimethylammoniobutanoate) metabolism and therapeutic administration of carnitine has been a major area of interest in patients receiving dialysis. In mammals, acyl groups are attached to carnitine via an ester bond at the third carbon in the cytoplasm. All functions of carnitine involve the esterified molecule translocating from one cellular compartment to another. During this process long chain acylcarnitine moieties can be removed from the cytosol or medium chain acylcarnitine moieties from the peroxisomes (11). The movement of acylcarnitine moieties from one cellular compartment to another may increase the likelihood of oxidation. Long chain acylcarnitine moieties are known to have deleterious effects on cellular membranes and metabolic functions (12). Patients with renal failure may have increased concentrations of long chain fatty acyls or an alteration in carnitine metabolism due to their uremic status.

The primary source of carnitine in humans is exogenous intake of carnitine, lysine and methionine via food (13). Meat, poultry, and dairy products are reportedly high in total carnitine (14). Therefore, low intakes of these high protein foods may lead to decreased

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intakes of carnitine. This has been shown in individuals on lactoovovegetarian diets (15), as depicted by lower serum carnitine concentrations. Although the serum concentrations of total carnitine were not clinically abnormal, they were significantly lower than the meat eating controls. Dietary intake of carnitine may not be sufficient in CKD patients due to decreased intake of high protein foods.

Treatment with oral and intravenous L-carnitine (biologically active form of carnitine) has been effective in increasing plasma total and free carnitine concentrations in CKD patients (16-18). However, the results have varied in clinical endpoint benefit.

Justification and Aims

Due to alterations in dietary carnitine intake, carnitine losses through dialysis treatment, and changes in carnitine metabolism in CKD, it is crucial to include carnitine metabolism assessment in the management of dialysis patients. Identifying CKD patients receiving dialysis who are at greatest risk for increased fatty acyl concentrations, or identifying risk factors for altered carnitine metabolism would facilitate determination of treatment strategies including exogenous L-carnitine. Accordingly, clinically significant benefits can be evaluated for the overall better health and quality of life of CKD patients receiving dialysis. This research was conducted to: 1) identify risk factors associated with altered carnitine status; 2) to determine the effect of L-carnitine administration in a sample of hemodialysis patients selected using the previously determined risk factors on specific clinical parameters and on plasma acylcarnitine moieties.

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Aim 1:

To determine risk factors for suboptimal plasma carnitine concentrations and associated factors in hemodialysis patients.

a. Hypothesis 1: Plasma carnitine alteration varies with individual patients and clinical variables correlated ($p<0.05$) with plasma total, free, short chain and long chain acylcarnitine will identify patients at greatest risk for altered carnitine metabolism.

Aim 2:

To determine if CKD patients receiving hemodialysis at increased risk for altered carnitine metabolism will respond to L-carnitine with regard to clinical parameters and perceived quality of life.

a. Hypothesis 2: Based on the premise that L-carnitine will increase oxidation of mitochondrial long chain acyl carnitine and increase mobilization. It was hypothesized that a statistically significant improvement will occur in clinical parameters (including perceived quality of life) correlated with plasma total, free, short, and long chain acylcarnitine.

Aim 3:

To measure the effect of treatment with intravenous L-carnitine on plasma acylcarnitine moieties in CKD patients who are receiving hemodialysis and at risk for altered carnitine metabolism.

a. Hypothesis 3: Treatment with intravenous L-carnitine will increase oxidation of mitochondrial long chain acylcarnitines and increase mobilization of medium and long chain acylcarnitines out of the mitochondria leading to a

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In order to accomplish the aims set forth, it was determined that it would be necessary to conduct a two phase study as follows:

Phase I – a cross sectional, observational study in which data were obtained from a medical record review and patient examination and interview in order to determine risk factors for suboptimal plasma carnitine concentrations and associated factors in hemodialysis patients;

Phase II – a randomized, double-blind, placebo controlled clinical trial in which data were obtained from blood collection (plasma), patient examination and interview, and medical record reviews. Patients selected based on defined criteria, were separated into treatment or placebo groups. The treatment group received 20mg/kg/treatment and the placebo group received a normal saline infusion. The purpose of this section of the study was to determine if CKD patients at increased risk for altered carnitine metabolism will respond to: 1) determine if CKD patients at increased risk for altered carnitine metabolism will respond to L-carnitine with regard to clinical parameters and perceived quality of life and 2) measure the effect of treatment with intravenous L-carnitine on plasma acylcarnitine moieties in CKD patients at risk for altered carnitine metabolism.

The organization of this dissertation is as follows:

- Chapter 2 – Review of Literature, with a complete description of CKD, dialysis, and carnitine as it pertains to CKD;
- Chapter 3 – Overview of methods used in phases I and II;

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- Chapter 4 – Manuscript 1, phase I - aim 1;
- Chapter 5 –Manuscript 2, phase II - aim 2;
- Chapter 6 – Manuscript 3, phase II - aim 3;
- Chapter 7 – Summary, strengths, limitations, and future research directions.

Chapters 4, 5, and 6 focus on the study aims. Chapter 4 focuses on the baseline correlations between plasma total, free, and acylcarnitine concentrations, acyl-to-free carnitine ratio and links to specific clinical parameters (age, weight loss, average monthly synthetic erythropoietin and mannitol dose, triglyceride, blood urea nitrogen, and parathyroid hormone concentrations, exercise tolerance, perceived quality of life, dietary intake, duration of treatment and treatment time, medications and co-morbidities). This phase was observational with no intervention occurring. Because phase I showed that patients who were older, female, consumed less dietary protein, had more arterial dilation, lower ejection fraction percentage, used aspirin and did not use mannitol were more likely to have lower plasma carnitine concentrations, it was determined that a carnitine intervention study would clearly establish whether or not carnitine might be beneficial when these factors are present. Therefore, a randomized, double-blind, placebo controlled clinical trial was designed for phase II, in which quality of life, synthetic erythropoietin dosage, muscle cramping and nutritional parameters were monitored for changes after treatment with L-carnitine. The timeline for both phases I and II is outlined in Table 1.2.

In Chapter 5, the results of phase II, aim 2 are reported. Variables such as age, duration of treatment, gender, medication use, and presence of disease (such as type 2 diabetes) were

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regarded as risk for altered carnitine metabolism. When patients from phase I were grouped by these selected risk factors, the mean carnitine concentrations for plasma total,

Table 1.2

Phase I and Phase II Timeline

Phase I				Phase II		
1/2000	5/2000	7/2002	8/2002	11/2002	2/2002	5/2002
Data collection	Data analysis: Abstract submission	Baseline data collection	Intervention period began	12 week data collection	24 week (final) data collection	Data analysis complete: Abstracts submitted

free and acylcarnitine decreased as the number of risk factors increased. Chapter 6 describes the effects of L-carnitine treatment on plasma acylcarnitine concentrations and on the plasma acetyl to linoleic acid ratio.

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Chapter 2

Review of Literature

Chronic Renal Failure

The kidney has many functions including a role in maintenance of water and electrolyte (eg: potassium, sodium, chloride, calcium and phosphorus) balance, regulation of acid-base balance, and removal of nitrogeneous waste products, primarily urea. Other functions of the kidney are to produce renin, erythropoietin, and 1,25-dihydroxycholecalciferol. However, a major function of the kidney is to produce urine. It does this by forming ultrafiltrate from the plasma in the glomeruli and proximal tubules. In the proximal section of the tubules a portion of sodium, chloride, and water is removed concentrating the ultrafiltrate. Other solutes such as potassium, magnesium, and calcium are totally or partially reabsorbed. As the ultrafiltrate continues through the Loop of Henle, distal convolution, and collecting ducts, the ultrafiltrate is refined by regulation of water, electrolytes, and hydrogen ions.

When a patient begins to lose renal mass he or she begins to adjust to this loss through an aggregate of mechanisms known as compensatory renal hypertrophy (1). In the early stages of kidney failure, the kidney begins to increase in weight and cross-sectional surface area. This increase in surface area is due to enlargement of glomeruli and proximal convoluted tubules. This enlargement takes place for the most part in the cortex and minimally in the medulla. As the patient continues to lose renal mass, each nephron that remains must excrete more water and solutes in order for homeostasis to continue.

However, nephrons have limits and eventually this loss of renal mass results in irreparable kidney damage (1).

Glomerular filtration rate (GFR) is one method to measure the progressive loss of renal mass and kidney function. In the clinical setting, the most common method for determining GFR is by comparing the amount of creatinine in urine to the amount measured in the plasma. Normal GFR for an adult human is 125mL/minute. However, as renal function declines, the amount of water and solutes filtered begins to decline until it reaches approximately 10 to 15 mL/minute (2). At this level of renal function, it is common for some kind of renal replacement therapy to begin in the form of dialysis. Previously referred to as end stage renal disease; however, the term commonly used now is CKD Stage 5 (3). The three most prevalent diseases leading to dialysis treatment in 2002 were diabetes mellitus, 45%; hypertension, 27%; and glomerulonephritis, 9% (4;5). There are two basic types of dialysis. The first, hemodialysis, is done three to four times per week and requires an internal or external vascular access. The second type of dialysis is peritoneal dialysis, which is conducted daily, within the peritoneal cavity of the patient. This study focuses on patients receiving hemodialysis. Hence, a more in-depth description of this type of dialysis is provided.

Hemodialysis

Hemodialysis is defined as "the removal of certain elements from the blood by virtue of the difference in the rates of their diffusion through a semi-permeable membrane, for example, by means of a hemodialysis machine or filter" (3). The process begins by

placing an access into the blood stream via a central line (e.g. subclavian or internal jugular) or an arterio-venous fistula. Blood is routed out of the body through a pump, which can be set at different speeds depending on the capability of the access, into a filter. The filter is in a "bath" of dialysate containing a mixture of ions such as calcium, potassium, and magnesium. Due to hydrostatic and oncotic pressure exerted by the dialysate and filter urea, sodium, potassium, phosphorus, excess fluid and some other water-soluble molecules such as albumin and carnitine are removed from the blood. The blood is returned to the body via the access. Hemodialysis treatment is typically done three times a week. However, this may vary for very small or very large individuals from as little as one time a week to as much as daily. Approximately 70% of plasma free and acetyl carnitine is lost through the hemodialysis membrane every time the patient is dialyzed (6).

Carnitine

Carnitine is a compound synthesized from the amino acids lysine and methionine in the kidney, liver, and brain. Approximately 75% of total body carnitine needs originate from food sources of carnitine, lysine, and methionine. The other 25% is generated from metabolic conversion. Unlike other tightly regulated nutrients (i.e. calcium, iron, sodium, potassium) in healthy adults, there is an association between dietary intake of carnitine and plasma carnitine concentrations ($r = 0.64$, $p<0.05$) (7). The status of carnitine in humans varies by body composition, gender, and overall diet. In lean adults when carnitine intake is restricted, plasma total and free carnitine concentrations and urine free carnitine excretion decrease. However, when obese individuals are placed on a carnitine-

restricted diet, the decrease tends to be blunted (8). When comparing total carnitine in female and male adults, females tend to have lower plasma concentrations and lower urinary excretion than males (9).

Carnitine and Diet

Dietary carnitine is found primarily in foods from animal sources. However, lower amounts are found in grains, fruits, and vegetables. In Table 2.1, a list of foods with corresponding carnitine content generated from the literature is provided. The methodology used to analyze carnitine content of these food items was either via radioenzymatic assay, which has been well validated, or via the spectrophotometric method, which when used for biological samples may be inaccurate. Furthermore, the carnitine amounts listed are ambiguous since the state of the food items and specific details about the food such as raw versus cooked and if cooked, which cooking method are not provided.

Nutritional intake patterns, including diet quality, probably have a greater impact on total body carnitine content than carnitine content in single foods. Individuals with minimal to no animal protein intake, such as vegans (9-11) or people with predominately cereal based diets (12) have lower plasma carnitine concentrations compared to people consuming diets including animal proteins. Lombard et al. (9) reported that adult and children who were lactoovovegetarians and vegans, have lower concentrations of plasma free and

Table 2.1

Carnitine Content in Foods

Food Item	µmol Total Carnitine/100g food	Reference
Animal Products:		
Steak, prepared	525	(13)
Beef (tenderloin, shoulder, rump), raw	3691.4 – 4160.5	(14)
Ground Beef, prepared	300	(13)
Beef liver, raw	160.4	(14)
Chicken, prepared	60	(13)
Egg, chicken, prepared	5	(13)
Grain products (dry, unless otherwise specified):		
Cornflakes	59	(13)
Grits	51	(13)
Rice	44	(13)
Bread	10.9	(13)
Graham Crackers	5	(13)
Oat seedling (80-90 hour)	8.64	(15)
Wheat germ	7.41	(15)
Wheat seed	2.47	(15)
Oat seed	0.62	(15)
Barley seed	0	(15)
Fruit:		(15)
Applesauce	19.5	(13)
Pears	17	(13)
Orange juice	11	(13)
Peaches	10	(13)
Apple juice	8	(13)
Pineapple	6.5	(13)
Vegetables:		(13)
Tomatoes	18	(13)
Cauliflower	8.64	(15)
Asparagus	8	(13)
Peas	7.2	(13)
Green beans	5	(13)
Avocado	4.94	(15)
Spinach leaf, cabbage head leaf, carrot, potato	0	(13;13;15)
Legumes:		
Peanut seed (minus seed coat)	0.62	(15)

Dairy:		
Milk, cow (μmol/100g)	16.4	(13)
Milk, cow (μmol/100ml)	259.8*	(16)
Milk, human	50.0*	(16)

* analyzed by flow-injection analyses with an immobilized carnitine acetyltransferase bioreactor. Method based on spectrophotometric determination (17)

total carnitine and have lower urinary excretion of total carnitine compared to similar age groups with animal proteins in their diet. The small difference in plasma carnitine, although statistically significant is probably not biochemically or clinically significant due to the lack of evidence of pathophysiological consequences. However, children with kwashiorkor (due to a low protein intake) have been reported to have decreased plasma carnitine concentrations when compared with healthy controls. The plasma carnitine was positively correlated with long chain fatty acid oxidation (18).

Fat content of the diet has been reported to alter carnitine status as well. For example, healthy adult subjects fed a high fat, low carbohydrate diet have significantly higher concentrations of plasma total and free carnitine and higher urinary excretion of total, free and acyl carnitine by day 6 of the diet compared to subjects fed a low fat, high carbohydrate diet (10;11). The mechanism for this extremely interesting phenomenon is yet unknown. Table 2.2 outlines total carnitine content from a variety of nutritional intake patterns as reported in the literature for healthy and unhealthy individuals.

Table 2.2

Carnitine Intake in a Variety of Nutritional Intake Patterns

Nutritional Intake Pattern	Total Carnitine µmol/day	Analysis/Information Source	Reference
Far East Indian:		Calculated based on values	(12)
Middle income	92.5±12.3	from Rudman et al, 1977	
Low income	55.5±12.3		
L-Carnitine restricted diet	64.14±16.87	Radioenzymatic	(19)
Cirrhotic patients	29*	Spectrophotometric	(13)
Normal controls	410*		
Wisconsin adults		Calculated based on values	(7)
(normal omnivores):		obtained from personal	
Females	182.7±329.6	communications with P.	
Males	290.1±518.5	Borum	

* Standard deviation not provided by reference

Carnitine can also be synthesized from the amino acids lysine and methionine in the body. There are two sources of these amino acids, dietary and endogenous protein degradation. The amount of lysine and methionine in the diet can therefore affect carnitine biosynthesis, especially if the intake of carnitine is negligible, such as in vegans (20;21). In 75 individuals, Krajcovicova-Kudlackova et al. (20), demonstrated significant differences in plasma free carnitine, dietary methionine, and dietary lysine in

vegans/lactoovovegetarians and omnivores. Additionally, they found significant positive correlations between plasma free carnitine and dietary lysine, and plasma free carnitine and dietary methionine in vegans and lactoovovegetarians, but not in omnivores. Dietary lysine and methionine, although essential are distantly removed from the carnitine biosynthesis process as outlined below. It is surprising that these amino acids are correlated with plasma carnitine and warrants further investigation.

Micronutrients such as vitamin C, iron, pyridoxine and niacin are necessary for carnitine biosynthesis. If intake of these is insuficient, then carnitine status may be compromised (22-24). Plasma carnitine concentrations were examined in guinea pigs deficient in vitamin C. The vitamin C deficient state decreases butyrobetaine hydroxylation (25;26). However, the extent to which overall carnitine biosynthesis is affected is unknown. In iron deficient individuals, carnitine status was positively correlated with serum ferritin concentrations (23;27). As serum ferritin concentration increased to within normal values, the concentration of plasma carnitine also returned to normal. Pyridoxine intake does not have a large impact on carnitine biosynthesis. However plasma short-chain acylcarnitine decreased without a significant change in plasma total carnitine when human subjects were fed a pyridoxine restricted diet (28). While these studies give insight to the role of micronutrients in carnitine homeostasis, the key issue in body carnitine status appears to be the overall quality of the diet.

Absorption of dietary carnitine through the gastrointestinal tract depends on the oral load consumed (19;29;30). For instance, in adults a high load diet (>6g carnitine per day)

approximately 5 to 15% of the oral carnitine is absorbed. Conversely, in a low load diet (<1g carnitine/day) greater than 75% of the carnitine is absorbed. The unabsorbed portion is degraded by bacteria in the colon into trimethyl amine and γ-butrobetaine (19;29;30).

To date, there are no known dietary components which impair absorption of carnitine nor is there a known toxicity associated with the commonly ingested amounts dietary amounts of carnitine. Although carnitine is found in high protein foods and hemodialysis patients have elevated protein needs (0.8g protein/kg for healthy vs 1.2g protein/kg for hemodialysis, respectively), patients are often unable to consume these high protein foods due to poor appetites or aversions to meats associated with uremia (31).

Biosynthesis of Carnitine

In humans, the rate of carnitine biosynthesis is determined by the availability of trimethlyllysine and its ability to cross the mitochondrial membrane in order to reach the intramitochondrial site of trimethyllysine hydroxylase activity (32). In humans, trimethyllysine hydroxylase plays a role in carnitine synthesis regulation. The regulatory effect of trimethyllysine was demonstrated by Rebouche et al (21), when supplemental trimethyllysine was given to humans, and an 8-fold increase in carnitine biosynthesis occurred. Humans tend to synthesize approximately 1-2μmol/kg/day (19). Carnitine is found in all biological tissues, although ninety-seven percent is located in skeletal muscle (33).

In humans, 30 to 50% of exogenously and endogenously produced trimethyllysine is converted to carnitine; the remainder is excreted in the urine (21). Within the kidney, 98-99% of tubular resorption of free carnitine occurs, unless the transporters become saturated (19). Healthy subjects excrete carnitine at a rate of 5 μmol/kg/day, which, for an 80 kg person is 2800 μmol/week.

Carnitine synthesis is a multi-step process, which begins with the addition of a hydroxyl group to the third carbon of lysine by the enzyme trimethyllysine dioxygenase (hydroxylase) in the mitochondria of the kidney, liver, heart, muscle, and brain. This step requires 2-oxoglutarate, Fe^{2+}, and molecular oxygen as cofactors as well as vitamin C to maintain iron in the ferrous state (34-36). The product of this reaction is 3-hydroxy-N^6-trimethyllysine, and it appears to be the only step in which the enzyme is located in the mitochondria.

Conversion of 3-Hydroxy-N^6-trimethyllysine to 4-N-trimethylammoniobutanal and glycine is catalyzed by 3-hydroxy-N^6-trimethyllysine aldolase. This enzyme is located in the cytosol, with the highest activity found in liver (34). Pyridoxine is required for aldolase activity because it uses pyridoxal 5'-phosphate as a cofactor. 4-N-trimethylammoniobutanal dehydrogenase catalyzes the formation of 4-N-trimethylammoniobutanoate, also known as butyrobetaine, from 4-N-trimethylammoniobutanal using niacin in the form of NAD as a hydrogen acceptor.

4-N-trimethylammoniobutanoate enters the circulation and then enters the kidney and the liver through an active transport mechanism. In humans, the activity of butyrobetaine dioxygenase is significantly higher in the kidney (3-16 fold) than in the liver, while the activity in brain is 50% that of liver. In the kidney or liver cytosol, 4-N-trimethylammoniobutanoate is hydroxylated on the third carbon to form L-carnitine. This reaction requires Fe^{2+}, molecular oxygen and vitamin C for activity (34).

After L-carnitine is synthesized, it is transported through the circulation and taken up by other tissues through active sodium-dependent transport. This sodium-dependent organic cation transporter (OCTN2) (37) has been cloned and characterized. The OCTN2 transporter is probably responsible for carnitine transport into the tissues and is involved in tubular reabsorption of carnitine in the kidney. Recently, other transporters, OCTN1 (37;38); , OCTN3 (39), CT2 (40) and $ATB^{0,+}$ (41), have been identified to carry carnitine.

Carnitine is located in separate compartments of the body with differing rates of turnover (42) and the muscle tissue to plasma ratio in humans is high at 100:1 (37). Liver contains approximately 500 to 1000 nmol total carnitine per gram wet weight. Liver carnitine rapidly interacts with plasma carnitine and has a half-life of one to two hours. Alternatively, skeletal muscle which contains 3000 to 5000 nmol total carnitine per gram wet weight, does not readily communicate with plasma, and has a half-life of several days (42). Therefore, changes in carnitine content in liver rapidly appear in plasma, whereas changes in skeletal muscle content may not be as readily apparent in plasma. The two

primary forms of plasma carnitine are free and acyl.These forms can readily be converted from one to the other.

Carnitine Functions

Carnitine has many potential functions. The first has been well established as a transport molecule for long chain fatty acyls crossing the inner mitochondrial membrane. Carnitine acyl transferase I catalyzes the formation of acylcarnitine from fatty acyl-CoA. This process is regulated by malonyl-CoA. As malonyl-CoA concentrations increase, carnitine palmitoyl transferase-I becomes inhibited (43). Carnitine/acylcarnitine translocase enzyme then catalyzes the transportation of the acylcarnitine into the mitochondrial matrix (44) where carnitine is separated from the fatty acyl, so the fatty acyl can enter the β-oxidation pathway to be broken down to form ATP. Second, carnitine functions in exporting acetyl- and chain-shortened acyl products from the peroxisomes preserving the cellular CoA homeostasis (44). Finally, carnitine releases mitochondrial CoA from acyl-CoA, when the free CoA supply becomes limited, due to activation and subsequent accumulation of metabolites in the mitochondrion, allowing the CoA to be used for other functions (43). Normal plasma concentrations of total, free, short and long chain acylcarnitine established by Hoppel (45;46) and for total carnitine concentrations for men and women established by Borum (47) are depicted in Table 2.3.

Table 2.3

Normal Plasma Carnitine Concentrations

Type of Carnitine	
Total	46.1 ± 10μm/L males = 51.7±10.8μm/L females = 43.8±11μm/L
Free	36.7± 7.6μm/L
Short chain acylcarnitine	5.7± 3.5μm/L
Long chain acylcarnitine	3.7 ± 1.5μm/L

When compared with healthy subjects, patients with CKD are reported to have higher acylcarnitine concentrations and lower plasma free carnitine concentrations; whereas, the total carnitine concentrations remain within the normal range (48).

In healthy subjects, the most prevalent form of plasma acylcarnitine is acetylcarnitine. Following carnitine removal, coenzyme-A (Co-A) is attached to acetate and acetyl Co-A is formed. Acetyl Co-A is a substrate for many metabolic pathways and in a healthy population is the primary metabolite resulting from β-oxidation. However, in plasma of

CKD patients, increased amounts of medium and long chain acylcarnitine concentrations are reported in the literature (48-50). The increase in medium and long chain acylcarnitine moieties may be due to an alteration in β-oxidation and/or a decrease in excretion of these metabolites. Long chain fatty acyls have deleterious effects on cellular membranes and metabolic functions. Hypothesized mechanisms for the negative effects are 1. interference with sodium-potassium transporters in cell membranes (51) and 2. detergent effects on cellular phospholipid membranes (52).

Carnitine and Chronic Renal Failure

The exact role of carnitine in hemodialysis patient metabolism is unclear. Patients receiving hemodialysis are thought to have secondary versus primary carnitine deficiency (53). Secondary carnitine deficiency is associated with a less obvious alteration in biochemical markers (plasma free-, acylcarnitine and acyl-to-free carnitine ratio) than primary carnitine deficiency, and is typically caused by an underlying genetically determined inborn metabolic error, an acquired disease (e.g. chronic renal failure, Fanconi Syndrom) or an iatrogenic factor (53). On September 8-10, 2002, The National Kidney Foundation held a consensus conference on carnitine, which established the term dialysis-related carnitine disorder (DCD) to describe the symptoms associated with altered carnitine metabolism (54). The four main types of symptoms that have been reported in the literature as accompanying altered carnitine metabolism are: anemia hyporesponsive to synthetic erythropoietin, intradialytic hypotension, cardiomyopathy and skeletal muscle weakness (55). However, even though the term DCD and its

associated conditions were established, it is not completely accurate because it implies that dialysis is the cause of the altered metabolic state of carnitine. However, in reality the actual mechanism of the alteration is unclear.

Prior to patients requiring dialysis, they gradually lose the ability to produce and filter urine and eventually the patient produces no urine at all. As the glomerular filtration rate drops, the concentrations of plasma acylcarnitine begin to rise. Normally these acylcarnitine molecules are lost in the urine, but as urine production decreases, plasma acylcarnitine concentrations increase. Controversy exists on the cause of the altered plasma carnitine concentration. The question is whether an actual decrease in plasma carnitine concentrations occurs thus causing metabolic consequences in hemodialysis patients or the metabolic consequences are caused by a defect in one of the β-oxidation enzymes of the mitochondrial matrix.

In pre-stage 5 CKD patients, Rodriguez-Segade et al (56) showed a plasma acyl-to-free carnitine ratio that was significantly higher than healthy controls. This higher ratio indicates that not only are the concentrations of acylcarnitine higher with a subsequent ratio increase, but possibly the actual metabolism of carnitine may be altered in patients with uremia. In 1987, Ricanati et al (50) argued that the concentrations of muscle, liver and acylcarnitine derivatives for hemodialysis patients were within normal ranges. The patients in this study were also pre-stage 5 chronic kidney disease at the time of data collection, were therefore a comparable population. Later, Hoppel et al (49) showed that although the amount of plasma total and acylcarnitine was not different in hemodialysis

patients, the types of acylcarnitine did differ significantly. Data from these studies suggest that in the case of pre-dialysis patients, the plasma concentration of carnitine may not be as indicative of altered β-oxidation metabolism, as the type of plasma acylcarnitine.

Acyl-to-Free Carnitine Ratio, Free Carnitine, and Acylcarnitine in Dialysis Patients: In subjects without chronic renal failure, carnitine is excreted through the kidneys. Both plasma free and acylcarnitine pass into the urinary ultrafiltrate, but 99% of the free carnitine is preferentially reabsorbed in the tubules. Plasma acetylcarnitine, a 2-carbon acylcarnitine moiety is excreted at a rate four times greater than that of free carnitine (57). This increased excretion of acetylcarnitine may keep the acyl-to-free carnitine ratio low. Data on healthy adults from Hopple and Brass (45) suggest that a normal acyl-to-free ratio is 0.26. In pediatric patients, a plasma acyl-to-free carnitine ratio of $\geq$ 0.4 can define a carnitine deficiency (53). In dialysis patients the ratio is typically between 0.6 and 0.9 (58), which may be elevated (58).

When patients reach stage 5 CKD and begin dialysis, plasma free carnitine is lost. As a small, water-soluble molecule, carnitine passes through the dialysis membrane and accumulates in the dialysis fluid (33;44;58;59). The plasma free carnitine is lost through the dialysis membrane at a rate similar to acylcarnitine (about 70%), affecting the ratio of acyl-to-free carnitine in hemodialysis patients (6).

Plasma from patients with chronic renal failure contains elevated concentrations of acylcarnitine formed from accumulating carnitine metabolism intermediates at the expense of free carnitine (42). This occurs for example, when acetate is used as an alkalizing anion in the dialysate fluid. Acetate is activated for metabolism by coupling to free CoA-SH reducing the intramitochondrial concentration of the essential cofactor (47). CoA-SH is also required for the oxidation of 2-oxoglutarate in the citric acid cycle. Inadequate CoA-SH concentrations can slow the metabolism of acetate. In the presence of adequate carnitine, the transfer of fatty acids from acyl-CoA to carnitine, followed by an export of acylcarnitine to the cytosol can regenerate CoA-SH. If acetate overload has depleted the concentrations of free carnitine, then both CoA-SH depletion and acyl-CoA buildup will limit acetate metabolism, and the anion will increase evan higher.

Therefore, even when free plasma carnitine concentrations appear adequate, they may not be sufficient to handle the high acetate loads potentially experienced during hemodialysis. Currently, dialysate in most centers consists of predominately bicarbonate and minimal amounts of acetate. However, based on findings of Borum (47), a problem may still exist even with low acetate concentrations. Additionally, the total carnitine concentrations in tissues are reduced in some people receiving hemodialysis (42). When measured, free plasma carnitine concentrations, in hemodialysis patients appear to drop during dialysis the return to normal requires several hours (6;59).

Studies conducted with hemodialysis patients indicate that this population has the potential for low plasma concentrations of free-carnitine (52;60). Giovenoli had 26

patients, who had been receiving hemodialysis for a minimum of one year. When the patients were divided into separate groups for three different methods (oral, interdialysate, or intravenous) of L-carnitine administration, the baseline measures of muscle free carnitine were below normal at 24.2 μM/liter, 19.2 μM/liter, and 28.0 μM/liter, respectively. Normal concentrations of free carnitine in the muscle were set at 36.7 ± 7.6 μM/liter for men and women by Brass and Hoppel (45). Matsumura et al (52) found that 26 patients, who had been receiving hemodialysis for a minimum of one year, had an average plasma free carnitine concentration of 21.8 μM/liter at baseline. These sub-optimal concentrations may be contributing to defects in metabolism of long chain fatty acids in hemodialysis patients and hence symptoms, such as: fatigue, muscle cramps, poor muscle strength, poor appetite and decreased quality of life.

Recommendations and Implication for Carnitine in Chronic Kidney Disease

In the year 2002, L-carnitine was approved by the Centers for Medicare & Medicaid Services (CMS) for national reimbursement in hemodialysis patients with either erythropoietin resistance or chronic hypotensive episodes during dialysis treatment.

There are at least four possible key metabolic areas, for which carnitine treatment may have important clinical outcomes for hemodialysis patients. These are: 1) malonyl-CoA concentrations – due to its role in regulating carnitine palmitoyl transferase-I and thus its role in hepatic mitochondrial fatty acyl oxidation, 2) plasma free carnitine concentrations – the availability of this substrate could affect the rate of the carnitine palmitoyl transferase-I enzyme, 3) carnitine palmitoyl transferase-I – due to its role in forming

acylcarnitine compounds, and 4) plasma acylcarnitine concentration– due to the reported variety of acyl forms unlike normal controls in CKF patients (49).

1) Malonyl-CoA

Malonyl-CoA has regulatory affects on carnitine palmitoyl transferase-I (43). Therefore nutrients or hormones which affect malonyl-CoA may ultimately impact long chain fatty acyl oxidation or carnitine metabolism. Kilocalorie and protein intake impact malonyl-CoA concentrations. As kilocalorie intake decreases so do malonyl-CoA concentrations, which in turn causes the inhibitory effect of malonyl-CoA to be removed. The kilocalorie and protein requirements for hemodialysis patients are estimated to be between 30 and 35 kilocalories per kilogram and 1.2 to 1.4 grams protein per kilogram for those patients with a normal body weight (3).

I. Kilocalorie, Protein, Normalized Protein Catabolic Rate and Blood Urea Nitrogen

Kilocalorie (kcal) and protein consumption are important to assess associations with plasma total, free, acylcarnitine and acyl-to-free carnitine ratio. Kilocalorie intake can impact the insulin to glucagon ratio, which can affect the inhibitory action of malonyl-CoA on carnitine palmitoyl transferase, the enzyme responsible for combining the long chain fatty acyl and carnitine (61). Protein intake will be examined through the calculation of the normalized protein catabolic rate. When the normalized protein catabolic rate is calculated interdialytic rise in blood urea nitrogen, time between dialysis treatments, and urine urea nitrogen are used to determine the rate of protein turnover in the body (3). This measure can be an indicator for adequacy of protein consumption. Blood urea nitrogen is a laboratory measure used in renal failure patients to monitor the

serum uremic status. The accepted normal ranges for a patient with renal failure are 60 to 100 mg/dl (3). A high blood urea nitrogen value might indicate a number of situations, including a high intake of total or low biological value protein, a gastrointestinal bleed or inadequate dialysis. High protein foods are the primary sources of carnitine. Therefore, a diet with $\geq$ 1g protein/kg body weight in total protein could potentially have more carnitine than a diet with $\leq$ 1g protein/kg body weight.

II. Nutritional Status

Patients with CKD have an increased risk for protein-energy malnutrition. An inexpensive, noninvasive method of determining nutritional status is subjective global assessment. The subjective global assessment is a series of questions about appetite, bowel functions (e.g. constipation, diarrhea), daily activities, and weight changes combined with a brief physical examination. Each question and the physical examination are answered on a seven-point scale. The reliability and validity of this test have been shown in studies with a small sample size (62-64). Specifically, in hemodialysis patients subjective global assessment has been positively correlated with albumin, pre-albumin, body mass index and other well established indicators of morbidity and mortality (62;64-66).

2) Free Carnitine

Between ten and seventy percent of hemodialysis patients have been reported to have some type of malnutrition which might be accompanied by muscle mass loss given that the primary site for carnitine storage in the body is skeletal muscle (3). The diet is the

primary source of carnitine, especially high protein foods, hemodialysis patients, who are not consuming sufficient kilocalories and protein to maintain skeletal muscle, may not be consuming sufficient free carnitine to replace the reported carnitine lost in the dialysate. Hence, sub-optimal plasma concentrations of carnitine might be a serious consequence of the combination of these two factors. Borah et a l (67) demonstrated that hemodialysis patients who did not consume $\geq$ 1.0 grams of protein per kilogram could not maintain positive nitrogen balance.

Free carnitine is lost at a similar rate to acylcarnitine into the dialysate, compared to healthy patients. Acylcarnitine is lost at a greater rate (54). Therefore, patients who receive longer (greater than three hours) dialysis for each treatment and have received it for a longer duration (in months or years) may have a greater potential for free carnitine loss.

3) Carnitine Palmitoyl Transferase-I

Carnitine palmitoyl transferase-I may be affected, as previously mentioned, by malonyl-CoA and free carnitine concentrations. Additionally, long chain fatty acyl-CoA availability and/or free fatty acids may affect carnitine palmitoyl transferase-I by stimulating and inhibiting the enzyme (43). There has been some evidence to suggest high serum calcium ions due to increased concentrations of parathyroid hormone may interfere with carnitine palmitoyl transferase-I (68) as well.

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I. Parathyroid Hormone

Chronic Renal Failure patients have low concentrations of 1,25-dihydroxycholecalciferol (active vitamin D) because the final step of activation (hydroxylation of 25-hydroxycholecalciferol) occurs in the kidney. Low concentrations of 1,25-dihydroxycholecalciferol can trigger a number of events leading to hyperparathyroidism, including low serum calcium concentrations. In 1987, Smogorzewski et al. (69) demonstrated that chronic renal failure is associated with impaired oxidation of long chain fatty acids by skeletal muscle possibly due the lack of availability of carnitine or to an effect on the enzyme, carnitine palmitoyl transferase, responsible for the formation of the long chain fatty acylcarnitine complex. In a later study by the same scientists (68), it was hypothesized that a connection between parathyroid hormone and chronic renal failure existed. This hypothesis posits that elevated parathyroid hormone concentrations resulted in increased calcium ions in the skeletal muscle. These high concentrations of calcium in the molecule may interfere with the action of the carnitine palmitoyl transferase I and thus affect long chain fatty acyl oxidation. Perna et al. (68) used a calcium channel blocker and found a positive effect on carnitine palmitoyl transferase, when calcium concentrations in the skeletal muscle were controlled.

4) Acylcarnitine

The acylcarnitine concentrations may be elevated or altered in hemodialysis patients. Hoppel et al (49) found an increase in the various types (e.g. acylcarnitine with 14 carbons and 10 carbons) of acylcarnitines that was not found in healthy controls. In healthy controls ninety-five percent of the acylcarnitines were acetylcarnitine compared

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to chronic renal failure patients, who had a wide range of medium chain acylcarnitines and these differences have been hypothesized by Hoppel et al to be caused by an acyl-CoA dehydrogenase defect rather than by a carnitine deficiency (49).

Consequences of Altered Carnitine Metabolism

Clinical manifestations of carnitine metabolism may include but are not limited to: anemia, fluid retention and muscle cramping or muscle weakness, hypertriglyceridemia, cardiac defects and poor perceived quality of life measurements.

Anemia

Anemia in the hemodialysis patient is usually monitored by measuring hematocrit and hemoglobin concentrations. It is of special concern to the hemodialysis patient because as the kidneys fail they produce less erythropoietin. Erythropoietin is a glycoprotein whose function is to stimulate the division and differentiation of committed erythroid progenitors in the bone marrow. Therefore, a large percentage of hemodialysis patients receive synthetic erythropoietin to replace what is not being made. Epogen® is the Amgen Inc. trademark for epotin alpha, which is the proper name for recombinant human erythropoietin.

Long chain fatty acyls are hypothesized to inhibit Na/K ATPase activity of the red blood cell membrane. Labonia et al (51) showed that treating 8 hemodialysis patients with oral (1 gram/day) and intravenous (1 gram 3 times per week post-dialysis treatment) L-carnitine increased the Na/K ATPase activity ($p<0.05$). Matsumura et al (52) showed low

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plasma total ($r = -0.56$, $p<0.01$) and acylcarnitine ($r = -0.58$, $p<0.01$) concentrations were associated with accelerated erythrocyte osmotic fragility in twenty-six hemodialysis patients. Nikolaos et al (70) reported a significant reduction in deformability (influences microcirculation, tissue oxygen delivery and life span) of 15 hemodialysis patients who received 30 mg intravenous L-carnitine/kg/dialysis treatment for 3 months. Labonia et al. (51), Trovato et al. (71) and Nikolaos et al (70) showed increases in hematocrit with carnitine treatment. The mechanism for this is thought to be the stabilization of the erythrocyte membrane by facilitation of lipid uptake. Treatment of 31 adult hemodialysis patients with 1 gram L-carnitine intravenously at the end of each dialysis treatment for 6 months resulted in decreases in Epogen® doses ($p<0.05$) during the treatment period. When the L-carnitine treatment stopped the Epogen® doses increased (72).

Muscle Function and Fluid Retention

Mannitol is a diuretic which increases the osmotic pressure of the glomerular filtrate, inhibiting tubular reabsorption of water and electrolytes, and elevates blood plasma osmolality, resulting in enhanced water flow into the extracellular fluid (73). Mannitol is used during some hemodialysis treatments when a hemodialysis patient has a large interdialytic weight gain to aid in removing excess fluid without causing the cramping that normally occurs when large amounts of fluid are removed(73). Hypertonic saline solution can also be used for similar effect in hemodialysis patients. L-carnitine treatment has been postulated to aid in decreasing muscle cramping from fluid removal. Bellinghieri et al (59) was able to report a decrease in the number of patient reported cramps during dialysis ($p<0.05$) when 2 grams/day of oral carnitine treatment was used in

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14 hemodialysis patients for 60 days. This data, although interesting is not quantitative nor detailed. Therefore it is difficult to determine if the observed effect is attributable to L-carnitine.

A study on the effect of L-carnitine on type I and IIa skeletal muscles in hemodialysis patients, demonstrated that at baseline, all of the patients had some type of muscle atrophy (60). The patients were separated into three groups and each group received a different treatment regimen. The first group had 0.0725 mM/L of carnitine added to the dialysis solution, the second group received two grams L-carnitine orally and the third received two grams intravenously at the end of each dialysis session. After a twenty-four week treatment period there was a seven percent increase in the diameter of type I and IIa fibers, suggesting an increase in muscle strength. When muscle strength, pain and cramps were tested, muscle strength improved in 7 out of 16 patients, muscle pain regressed in 2 out of 5, and muscle cramps decreased in 6 out of 10 patients (60). All three groups showed improvements in muscle function. It was hypothesized that patients with higher carnitine concentrations would have a greater tolerance for exercise. A limitation was the lack of information regarding the patients exercise patterns before and during the study.

In 1998, Sakurauchi et al. (74) treated thirty hemodialysis patients with muscular weakness, fatigue or cramps/aches with intravenous L-carnitine. After twelve weeks of treatment, sixty-six percent of the patients had at least some improvement in muscular symptoms. This data was patient reported and no detailed information on in cramp

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intensity nor number of cramps per treatment was given. Therefore, the study findings need to be interpreted cautiously.

Hypertriglyceridemia

Long chain fatty acids have two main pathways for metabolism. The long chain fatty acids can use carnitine to enter the mitochondria and begin β-oxidation or the long chain fatty acids can be formed into triglycerides. If carnitine metabolism is abnormal, an accumulation of triglycerides may occur. Vacha et al. (75) treated twenty-nine chronic hemodialysis patients with twenty milligrams per kilogram of intravenous L-carnitine in a cross-over design study. Each group of patients received both placebo and treatment for one hundred and twenty days. Patients with high-density lipoprotein cholesterol concentrations below forty milligrams per one hundred milliliters had significant triglyceride lowering effects during the treatment period. Mean triglyceride concentrations werereduced from 365 ± 13 mg/100 ml to 181 ± 6 mg/100 ml ($p<0.001$) (75). In those patients with the high-density lipoprotein cholesterol values greater than forty mg/100 ml no significant change was seen with L-carnitine treatment.

A 1998 study by Elisaf et al (76), on 28 dialysis patients treated with 5 mg/kg body weight intravenous L-carnitine over 6 months, reported significantly lower triglyceride concentrations ($r = 0.34$, $p<0.05$). The results from this study might have been stronger if higher amounts of L-carnitine were used (i.e. 20 mg/kg body weight intravenously). Furthermore, high density lipoprotein cholesterol concentrations were not controlled for in the Elisaf et al (76) study which may be a limitation. Stefanutti et al (77) examined L-

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carnitine treatment effects on a population of hemodialysis patients with triglyceride values between 170 and 260 mg/dl and between 220-260 mg/dl. These patients showed significant decreases in serum triglyceride concentrations after 30 days of treatment with 1 g 3 times a day of oral L-carnitine ($p \leq 0.02$). The mean triglyceride values dropped from 270 ± 36.1 mg/dl to 249 ± 52.1 mg/dl (77), which is clinically significant, but not to within normal concentrations (≤ 220 mg/dl).

Cardiovascular Disease

Dialysis treatment has improved to a point where the treatment itself is not the main cause of mortality. Rather, secondary causes such as cardiac disease are major contributors to mortality in this population. Cardiac disease in the renal patient is probably multifactoral. Hemodialysis patients have symptoms such as hyperlipidemia, hypertension and left ventricular hypertrophy, which all contribute to cardiac disease. The heart muscle uses non-esterified free fatty acyls as an energy source and therefore needs carnitine to transport fatty acyls beyond hexoate (78). Defects in carnitine metabolism have been linked to cardiac disorders such as ischemic injury and cardiomyopathy.

Quality of Life

As treatment methods improve and the length of life on dialysis increases quantifiable measures of quality of life have become increasingly important in determining risk of mortality. Measurement of patients' perceived quality of life is therefore an important outcome assessment in CKDl. Many assessment tools are currently being used for CKD

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patients. Heacock et al (79) studied perceived quality of life using The Ferrans and Powers Quality of Life Index for associations with nutritional status in hemodialysis patients. In this study the researchers began to investigate the effect of nutritional status on the patient's overall quality of life (79). It showed that the more nutritionally sound (biochemical and anthropometric), the better the quality of life.

One of the better known tools, the Medical Outcomes Study Short-Form-36 (SF36), has been shown by Kutner and others to have good reliability and validity (80-82). Higher plasma concentrations of carnitine have been associated with better quality of life (42;72;83-85). Sloan et al (86) demonstrated that an increase in the physical and general well being components of the SF36 after oral treatment with 1g L-carnitine for 1 ½ months and Brass (83) reported an improvement in the fatigue portion of the Kidney Disease Questionnaire. Both of these findings suggest that L-carnitine might have a positive impact on perceived quality of life by the patient. However, Sloan et al (86) measured the SF36 every month and the improvement in the physical and general well being only occurred after the first month of treatment but disappeared for the remainder of the study. The lack of subsequent significant findings may be attributed to the methodology used in the study. The L-carnitine was administered orally. When L-carnitine is administered in this manner the gastrointestinal tract breaks the undigested portions down by bacterial degradation into γ-butrobetaine or trimethylamine, which are typically excreted in the urine (19;29;30). Patients with stage 5 CKD do not produce significant quantities of urine and therefore cannot excrete these by-products. Therefore,

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the serum concentrations of the products may increase to a detrimental level, and negate any beneficial effects the initial treatment of L-carnitine may have had.

The SF36 has 8 different sub-scales, known as domains, related to self-perception of health-related quality of life (Table 2.4). The range for each of the 8 domains is 0 to 100. The domains may be examined individually, more specifically those related to physical activity (physical function, role-functioning, general health and vitality) and the physical component score (Figure 2.1). It is beneficial to assess the individual scores and the composite scores to more specifically determine which areas the patient feels are sub-optimal in their life. Be determining the specific are which is low, subsequent interventions can be determined.

Table 2.4

Medical Outcomes Short Form 36 Domains

Scale	Low Score Indicators
Physical functioning	Limitations in physical activity, inc. bathing & dressing
Role-Physical	Limited work due to health
Role Emotional	Emotions, limited daily function, and work
Social Function	Physical & emotional signs/symptoms limit social activities
Bodily Pain	Severe limiting pain
Mental Health	Feels nervous & depressed all the time
Vitality	Feels tired & worn out all the time
General health	Health much worse than previous year

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Figure 2.

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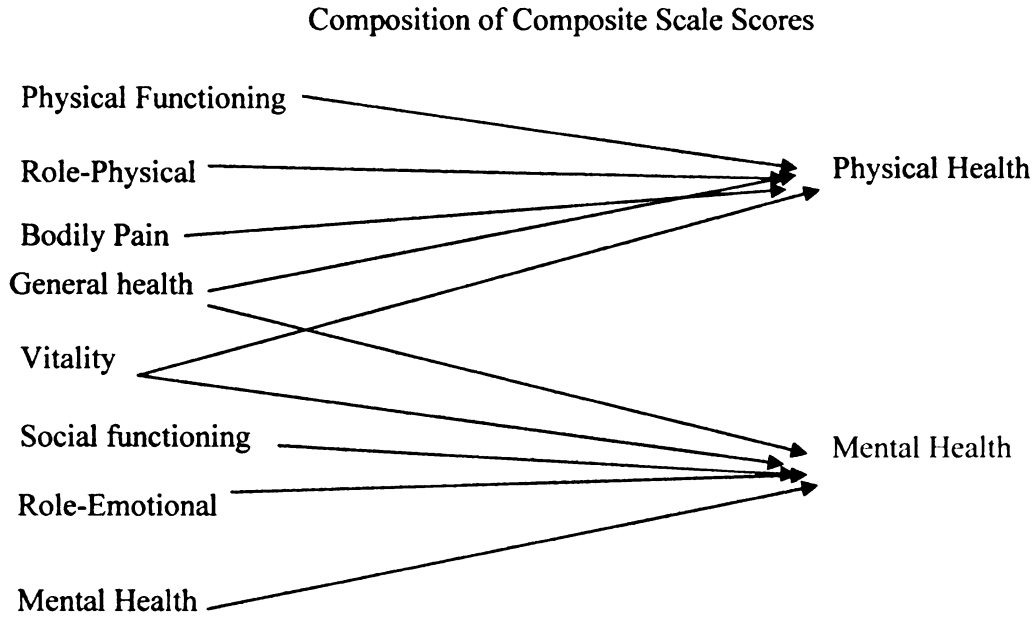

Figure 2.1. Domains included in the physical and mental composite scores

Carnitine Supplementation

L-carnitine can be given orally on a three times per day basis or intravenously via a catheter after dialysis treatment 3 times a week. The two methods of administering L-carnitine have potential positive and negative effects. First, oral administration can be inexpensive and effective. Daily, oral doses of L-carnitine of between 2 and 6 grams have been shown to have a systematic bioavailability on average of 18% (19). Oral L-carnitine may effectively carry carnitine to the liver during a "first-pass effect". Because carnitine functions as a carrier protein for long chain fatty acids, into the mitochondria for β-oxidation, the delivery of carnitine to the liver may facilitate the efficiency of oral L-carnitine in the reduction of serum triglycerides, by increasing the number of long chain fatty acyls, which cross the mitochondrial membrane and enter β-oxidation (42).

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Unfortunately, the following potentially negative attributes of oral L-carnitine could interfere with consistent delivery of L-carnitine to the hemodialysis patients. These could include patient compliance, and compromised absorption through a malfunctioning gastrointestinal tract. The researcher or clinician cannot control patient compliance. Compliance issues may include the number of tablets the patient chooses to consume and the number of times per day they choose to take the tablets. Many hemodialysis patients are on multiple oral drugs, and they could become overwhelmed. Furthermore, 45% of hemodialysis patients have diabetes (5) and 20-30% of these patients may have slightly or profoundly impaired gastrointestinal tracts due to gastroparesis (a complication of diabetes) so that the rate or amount of drug absorbed could vary from patient to patient (87).

Conversely, a dialysis nurse gives intravenous L-Carnitine at the end of the dialysis treatment, and therefore compliance is no longer an issue. Although the intravenous carnitine is only administered 3 times per week versus daily, intravenous L-carnitine administered in this way has been shown to have positive metabolic effects in studies (42;70-72;76;88). Because it is given directly into the blood stream, the gastrointestinal tract is completely bypassed, and consequently the exact amount of L-carnitine received by the patient is known. In 1998, Caruso et al (72) used 1 gm of intravenous L-carnitine 3 times per week in 31 hemodialysis patients with various etiologies for 6 months. These researchers were able to show a decrease in Epogen® doses (to improve anemia) and an increase in total and free plasma carnitine concentrations. Caruso et al (72) did not report the acyl-to-free plasma carnitine ratio, nor did they report whether the acyl-to-free plasma carnitine ratio decreased after supplementation with L-carnitine. Elisaf et al., (76) used 5

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mg per kg of body weight of intravenous L-carnitine 3 times per week, on 28 hemodialysis patients, for 6 months and induced a significant decrease in serum triglycerides of those patients who initially had above normal triglycerides. They also showed an elevation in plasma free and total carnitine after supplementation with L-carnitine. Elisaf et al (76) did not report any acyl-to-free carnitine ratios.

The Food and Drug Administration has approved both oral and intravenous L-carnitine for the treatment of both carnitine abberations. However, only intravenous L-carnitine has been approved for use in hemodialysis patients. It is well tolerated with no major side effects reported (13;89-91).

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Chapter 3

Overall Methods

This study was conducted in two phases; phase I was a cross sectional, observational study and phase II was a randomized, double-blind, placebo controlled, clinical trial. Phases I and II were conducted at facilities owned by the Dialysis Center of Lincoln (DCL), in Nebraska. These sites were Lincoln, NE (2 sites), Beatrice, NE (1 site) and Columbus, NE (1 site). Approvals to conduct the studies were obtained from the following (Appendix A):

1. University Committee on Research Involving Human Subjects (UCRIHS) at Michigan State University, East Lansing, MI
2. Community Institutional Review Board in Lincoln, NE
3. The Administrator at DCL
4. The Medical Director of DCL

Patients who participated in either phase had an informed consent read to them and/or read it themselves, were given the opportunity to ask questions, and were then asked to sign the consent (Appendix B) if they agreed to participate in the study.

Phase I sample

The following study inclusion criteria were used:

- patients $\geq$ 18 years of age
- receiving a minimum of four hours of bicarbonate hemodialysis treatments, at least three times per week
- have received dialysis treatment for a minimum of one year.

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Patients were excluded if they met the following criteria:

- currently or previously treated (within the last two months) with L-carnitine,
- had a severe blood loss within the past week prior to commencement of the study,
- had a disease affecting skeletal muscle function, severe liver disease and/or were pregnant.

These exclusion criteria were selected to minimize any confounding issues, which would alter carnitine metabolism beyond the effect of dialysis treatment or dietary intake. Therefore, patients with severe blood loss were excluded because they are typically given increased doses of erythropoietin and iron supplements to help increase blood volume. Patients who have diseases affecting skeletal muscle function were excluded because the disease may confound the correlation between carnitine and the exercise parameters that were investigated. Additionally, patients with severe liver disease and/or pregnancy may have altered carnitine metabolism for reasons other than uremia.

Phase I Data Collection

Data for phase I were collected by the researcher and a dietitian (MS, RD) working in the renal unit at DCL. The data were collected during the first week of January in 2000. Table 3.1 outlines all the data that were collected.

Table 3.1

Variables Collected in Phase I

Variable	Format Collected	Units
Total, free, short and long chain acylcarnitine	Plasma, pre-dialysis	μmol/L
Triglycerides Blood urea nitrogen Parathyroid hormone Hematocrit	Serum, pre-dialysis (all)	mg/dl mg/dl pg/ml %
Weight status	Digital Scale, post-dialysis	Kg
Treatment time/dialysis treatment	Medical record, actual treatment time	Minutes
Erythropoietin dose	Medical record	Mean unit/month
Mannitol dose	Medical record	Mean dose/month
Medication use: aspirin, lipid lowering	Medical record	Currently taking: yes/no
Time from initial dialysis treatment	Medical record	Years
Age	Medical record	Years
Exerciser Perceived dyspnea Perceived exertion	Medical record	Yes/no Scale 1-20 Scale 1-20
Perceived Quality of Life: Medical Outcome Short Form- 36	SF36 Questionnaire	1-100, 8 domains
Diabetes Mellitus Cardiovascular disease Left ventricular hypertrophy Left arterial dilation	Medical Record	Presence thereof indicated by yes/no for each co-morbidity
Dietary protein and kilocalorie intake	24 hour and typical day recall	Mean g/day and kcal/day, reported as total and per kg body weight

Dependent Variable Definition

The dependent variable for this study was plasma carnitine (total, free, short and long chain acylcarnitine). A Registered Nurse (RN) or the phlebotomist at DCL drew the participating patient's blood on a Wednesday or Thursday of the first week of the month

in accordance with the patient's routine monthly blood draw. No patient had been without dialysis prior to the blood draw for longer than 48 hours. A 1 ml aliquot of plasma from the blood sample was collected in a separator tube and frozen at –80° C until it could be analyzed. The plasma was analyzed for free, short and long chain acylcarnitine using the radioenzymatic assay as outlined by Brass and Hoppel (1). Total carnitine was calculated as the sum of the free, short, and long chain acylcarnitine values.

Independent Variable Definition

Weight and height, all medication use and dose, age, exercise patterns and tolerance, treatment information, and presence of co-morbidities data were collected from patients' medical records (some paper and some computerized). Weight and height were used to calculate body mass index and body weight was used to express kilocalorie (kcal) and protein intake per kilogram (kg).

Information on medication use was collected specifically related to lipid lowering medications, due to their effect on serum triglycerides and their potential to confound plasma carnitine and serum triglycerides concentrations. Additionally, consideration was given to medications that might affect cellular acetyl Co-A concentrations such as aspirin. Synthetic erythropoietin dosage was collected based on previous literature, which suggested an association between the prescribed dose and plasma carnitine concentrations (2).

Data on co-morbidities such as diabetes, cardiovascular disease, and hypertension were collected not only to describe the sample analyzed, but also to provide information on what disease states might or might not affect carnitine status in the sample. The data on left ventricular hypertrophy (LVH) and left arterial dilation (LAD) were obtained from the medical chart based on findings from echocardiography conducted no more than 6 months previously. Not all patients had this information. Therefore, data from only a subset of patients were analyzed for LVH and LAD.

Laboratory data were extracted from routine monthly laboratory profiles of each patient. These serum markers were obtained at the same time the carnitine samples were drawn. The serum was analyzed by Spectra Laboratories (Fremont, California). Biomarkers such as serum blood urea nitrogen and serum albumin were determined to assess nutritional status, as well as serum triglycerides (lipid metabolism), and hematocrit (iron status). Parathyroid hormone concentrations were assessed because previous literature (3-5) indicated associations between these variables and plasma carnitine concentrations.

Patients who are dialyzed at DCL are given a "prescription" to exercise when they initially begin their treatment at the facility. This prescription is a doctor's order for an exercise physiologist to assess a patient's exercise capacity and make an exercise recommendation. For the majority of patients, this entails riding a stationary bike while receiving dialysis treatment. The stationary bike is attached by chains to the lower part of the dialysis chair. On the day of data collection, patients who biked during their dialysis treatment were considered "exercisers". The rate of perceived exertion and perceived

dyspnea were used to define exercise tolerance (6). Both of these scales use a 1 to 20 rating with 20 as the most exertion or dyspnea and 1 the least. Patients who bike do so almost every dialysis session. Each time a patient bikes, the rate of perceived exertion and perceived dyspnea as reported by the patient are recorded into the computerized record.

Measurement of patients' perceived quality of life is an important outcome assessment tool. The SF36 has eight different scales related to self-perception of health-related quality of life. The range for each of the eight scales is 0 to 100. From the 8 domains, weighted means are calculated to form 2 composite scores: physical and mental. The SF36 was administered and scored by trained social workers from DCL, and the most recent results were used (Appendix C). If an SF36 had not been done in the past six months, it was done on the day of blood draw by one of the social workers. Patients at DCL are given the SF36 biannually, and are therefore familiar with the tool.

During the week of the blood draw, two dietary recalls (24 hour and usual day) were conducted on each patient participating in this study by either the dietitian at DCL or the investigator. The completed recalls were analyzed for average kcal and protein content and presented as both a total value and as the total divided by the patients' weight in kg. Patients of differing weight have differing kcal and protein requirements. Therefore, the total intakes were divided by weight to standardize the intake between all patients. Analysis of the dietary recalls was done using the software program Nutritionist V produced by N^2 Computing, 1999.

Phase I Data Analyses

Descriptive statistics were done on both the independent and the dependent variables. The continuous independent variables were assessed for associations with the dependent variables: total, free and acyl (short chain acyl + long chain acyl) plasma carnitine concentrations using the Pearson's Correlation Coefficient unless the variables were skewed, then Spearman's Correlation Coefficient was used. Each variable was plotted to determine whether it was normally distributed or not. Patients were separated based on gender, mannitol use, presence of diabetes (type 1 or type2) for analysis. Independent t-test analyses were done for the binary variables to determine associations with the dependent variables and the continuous independent variables. Independent t-test analyses were also used to compare mean protein and energy intake between patients treated or not treated with mannitol. To determine whether any of the independent variables were predictors (when controlling for potential confounders) of the dependent variables, multiple linear regression was used. Since the purpose of this study was to identify important variables for the next phase of the project. correlations between outcome variables typically used in dialysis care and carnitine concentrations were deemed significant using a p value of less than 0.05.

Phase II Sample Recruitment

This was a prospective, randomized, double blind, clinical research study. The inclusion criteria for phase II were:

- patients $\geq$ 18 years of age,

- receiving a minimum of three hours of bicarbonate, low acetate hemodialysis treatments, three times per week, for at least one year,

Based on phase I study findings, patients admitted into the study needed to have two or more risk factors for a compromised plasma carnitine status (Table 3.2).

Table 3.2

Risk Factor Criteria

1. 65 years old
2. 1 year duration of treatment
3. female
4. use of aspirin and/or mannitol
5. presence of type 2 diabetes mellitus, left atrial dilation and/or left ventricular hypertrophy

The exclusion criteria below were established on the same basis as in phase I, to minimize confounders:

- current or previous treatment (within the last two months) with L-carnitine,
- a severe blood loss within the past week prior to commencement of the study,
- disease affecting skeletal muscle function,
- severe liver disease,
- pregnancy

The Federal Drug Administration has approved intravenous L-carnitine for use in the hemodialysis population if plasma free carnitine concentrations are less than 40 μmol/L. Therefore, when a patient's plasma free carnitine concentration was greater than 40

μmol/L the patient was excluded from the study. Patients included into this study signed a consent form and were randomized into control and treatment groups.

Phase II Data Collection

The variables and data collection points are outlined in Table 3.3. The researcher and a senior level nutrition student from Michigan State University collected data. At each data collection time, they flew to Lincoln, NE and conducted patient recruitment, patient interviews and physical examinations, medical record reviews and arranged for blood collection and transport to the laboratory of Dr. Alan Davis in Grand Rapids, MI. For any patients who were not available at the time of their visit, the renal dietitians who worked at DCL facilities collected the missing data.

Variable Definition

Patients had their blood drawn for **plasma carnitine** analysis during the study on the first week of the baseline month, the 12th week and the 24th week in accordance with routine monthly blood draws. These periods for data collection were selected after an extensive review of the literature revealed these to be the most common data collection points. A renal registered nurse or dialysis technician collected a 1 ml aliquot of plasma from the blood sample in a heparinized tube. The blood sample was spun, the plasma was transferred via a pipette into a new test tube, and then the plasma was frozen to -80 degrees. The plasma was sent packed in a sealed polystyrene container on dry ice to the Spectrum Laboratory in Grand Rapids, MI, for determination of **total and free carnitine** using the radioenzymatic assay outlined by Brass and Hoppel (1;7). In this laboratory,

when the samples of plasma were thawed for analysis. A portion was separated, refroze, and sent to Dr. Charles Hoppel's laboratory at the Louis Stokes Veterans Administration Medical Center in Cleveland, OH for analysis of both **acid soluble and insoluble acylcarnitines** using high performance liquid chromatography with mass spectrometry (8).

Table 3.3

Data Collection Format

	Baseline	Monthly	12 weeks	24 weeks
Carnitine:				
Total	X		X	**X**
Free	X		X	**X**
Acyl (FA Lengths)	X		X	**X**
A/F ratio	X		X	**X**
Kcal/protein intake/nPCR	X		X	**X**
Subjective Global Assessment, MAMC	X		X	X
Short-Form-36	X			**X**
BUN, Hct, Hgb, TG, Alb, PTH*, HDL*	X			**X**
Mean Epogen® dose		X		
IDWG, mannitol/hypertonic	X		X	**X**
Dialyzer type, Aspirin use	X			**X**

Bold variables = primary clinical outcomes
Abbreviations: A/F ratio=acyl to free plasma carnitine ratio, Kcal=kilocalories, nPCR=normalized protein catabolic rate, MAMC=mid-arm muscle circumference, BUN=serum blood urea nitrogen, Hct=hematocrit, Hgb=serum hemoglobin, TG=serum triglycerides, Alb=serum albumin, PTH=serum parathyroid hormone, HDL=high density lipoproteins, IDWG=interdialytic weight gain, ASA=aspirin use

Two dietary recalls were collected using the multiple pass method (9), a 24 hour recall and a typical day 24 hour recall. The Multiple Pass Method of conducting a 24 hour

recall was developed by the United States Department of Agriculture for the National Health and Nutrition Examination studies. This technique was used to gather recall assessment data in phase II because of its increased accuracy (9). The recalls were analyzed using Nutritionist Pro., produced by First Databank, 2002. The **total kcal and protein** values from the recalls were averaged in order to account for individual variations and presented as total kcals, total protein (g) or kcal/kg and protein g/kg

Normalized PCR is a calculated valued used to assess total body protein turnover and dietary protein intake in hemodialysis patients. The following equation is used to calculate nPCR. In this study, the association of nPCR and plasma carnitine is examined in conjunction with actual dietary protein intake.

$$PCR = 0.22 + 0.036 \times \frac{\text{interdialytic rise in BUN} \times 24}{\text{interdialytic interval in hours}}$$

To normalize the PCR: nPCR = PCR/weight (kg)

As discussed in the review of literature (Chapter 2), **SGA** is a nutritional assessment tool recommended by the National Kidney Foundation's Guidelines for practitioners in dialysis (Kidney/Dialysis Outcome Quality Initiative). In phase II, the format for SGA was changed. Instead of using the A, B, C method originally presented by Detsky et al (10), as was done in phase I, the seven point SGA form designed by Linda McCann (11) was used (Appendix C). This form allows for greater flexibility in scoring. In small validation studies, it has been shown to have higher correlations with nutritional markers such as albumin and mid-arm muscle circumference (MAMC) (12;13). The SGA is a subjective measure. Therefore, in order to obtain more objective data on body

composition, the **MAMC** (14) was used to assess lean body mass. Mid-arm muscle circumference is calculated from the mid-arm-circumference and tricep skin fold measurements. While tricep skin fold is an indicator of body fat, the MAMC is used as an indicator of lean body mass by comparing the results to the findings reported by NHANES. The MAMC is inexpensive and relatively simple to conduct.

The **SF36** is a perceived quality of life assessment, which has eight domains used to comprise either a mental or a physical composite score. Each domain was scored on a scale of 0 to 100, with 100 being the highest perception of functioning. The domains are physical functioning, role-physical, bodily pain, general health, mental health, role-emotional, social functioning, and vitality. The SF-36 surveys were administered and scored by the social workers at DCL at baseline and at week 24 of the study, (Appendix C).

Data for laboratory parameters (**blood urea nitrogen, serum triglycerides, serum albumin, hematocrit** and **hemoglobin, high density lipoproteins** and **parathyroid hormone**) were taken from the monthly reports routinely collected by the facilities.

Erythropoietin doses, muscle cramping data, hypertonic saline or mannitol doses, and pre- and post-dialysis weights were collected from the Nursing Flow Sheet recorded by the dialysis technicians or nurses at each dialysis session (Appendix C). Disease etiologies and co-morbidities, initial dialysis treatment data, treatment duration and

procedures, medications, height, age, gender, were collected from computerized medical records at DCL.

In both phases, random data audits were conducted to ensure consistency and accuracy of collection and database entry. For example, in every 5th or 6th patient a second SGA was performed by one of the other data collectors. Results from the two assessments were compared. If significant variation occurred, a third assessment was conducted. This same procedure was followed for MAMC and 24 hour recall data. Additionally, when data were entered into a Microsoft Excel spreadsheet and SPSS software, random checks with the original data were conducted for accuracy.

Phase II Intervention Procedures

Patients eligible to participate in the study were randomized into either the treatment or the control group by a co-investigator who did not participate in the data collection process. This investigator and the pharmacist at DCL were the only persons not blinded. Those patients who were in the treatment group received 20mg Carnitor® per kg actual body weight, post hemodialysis treatment, three times per week intravenously. The carnitine was administered to the patients in an infusion of 100ml of normal saline over 10 minutes by the dialysis center nursing staff. The patients in the control group received an intravenous placebo of 100 ml normal saline over 10 minutes. The amount of carnitine needed was calculated using the patients' dry body weight determined at baseline of the study. By diluting the carnitine in normal saline and giving it in an infusion, the potential odor of the drug was masked and blindness maintained. The treatment period was for 24

weeks. In accordance with UCRIHS at Michigan State University and Sigma Tau Pharmaceuticals, Inc., the providers of L-carnitine, adverse events were monitored by the nursing staff and reported by the researcher to the University Committee for Research in Human Subjects and Sigma Tau Pharmaceuticals.

Phase II Data Analysis

These data were analyzed using SPSS version, 11.0, 2001. The change in clinical outcomes from baseline to post-intervention for each group of patients was examined by dependent t-tests and repeated measure analysis. Dependent t-tests can measure differences in mean values between groups that are connected in some manner such as one variable collected at two different time periods on the same person. Repeat measure analysis allows all the data to be placed into the analysis and analyzed for time and group effect. Associations between dependent and independent variables were determined by Pearson's correlations or Spearman's correlations depending on the nature of the variable. Some of the variables were analyzed for multivariate relationships with multiple linear regressions to determine predictability of the independent variables for the dependent variable.

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Chapter 4

Phase I, Aim 1

Carnitine Concentrations and Clinical Outcome Parameters

In Chronic Hemodialysis Patients

Steiber AL, Weatherspoon LJ, Spry L, Davis AT. *Clinical Nutrition* (2004) 23, 27-34

Abstract

Carnitine metabolism and the therapeutic use of carnitine has been a major area of interest in dialysis patients. The purpose of this study was to determine whether any correlations exist between carnitine status and selected clinical parameters in hemodialysis (HD) patients. This study was an observational study of data from patients receiving HD at a Midwest dialysis center. The subjects (n=49) were 60±16 (mean±SD) years of age and 48% male. Fifteen percent of the subjects had type 1 diabetes mellitus (DM), 29% had type2 DM, and 25% had left ventricular hypertrophy (LVH). The serum free and total carnitine, and acylcarnitine concentrations were: 40.3±11.8 μmol/L, 22.8±7.3 μmol/L, and 17.5±5.9 μmol/L, respectively. The serum acylcarnitine to free carnitine ration (A/F) was 0.80±0.27. Serum blood urea nitrogen (BUN), parathyroid hormone and ejection fraction were positively correlated and age and left atrial dilation (cm) were negatively correlated with serum total carnitine ($p<0.05$). BUN and hematocrit were positively correlated ($p<0.05$) and age was negatively correlated with plasma free carnitine. Subjects who used mannitol or were male had significantly higher concentrations of both plasma free and total carnitine, respectively ($p<0.05$). Subjects using aspirin had lower concentrations of serum total carnitine ($p<0.10$). These results suggest certain subgroups of patients may need to be targeted for further studies with

carnitine replacement therapy, i.e. long-term patients, older patients, patients with left ventricular hypertrophy and left atrial enlargement, females and patients on aspirin therapy.

Introduction

Carnitine has been shown to be beneficial in the treatment of uremic manifestations of dialysis patients such as anemia, fluid retention, muscle weakness, and poor quality of life scores (1-9). Seventy-five percent of carnitine in the body is ingested and the other 25% is synthesized in the kidney, brain, and liver. In adults, the source of carnitine is primarily red meats and in infants, human milk (10). Although carnitine is found in high protein foods and hemodialysis patients have elevated protein needs (11), the patients are often not able to consume sufficient amounts of these high protein foods to meet their needs due to poor appetites or aversions to meats caused by uremia.

The primary function of carnitine is to transport long-chain fatty acyl-CoA into the mitochondrial matrix. Other functions of carnitine are exporting products of β-oxidation from peroxisomes and releasing mitochondrial CoA from acyl-CoA when free CoA supply is limited.

Carnitine acyl transferase I catalyzes the formation of acylcarnitine. The carnitine/acylcarnitine translocase enzyme catalyzes the transportation of acylcarnitines into the mitochondrial matrix (12) where they can be transacylated to acyl-CoA. These acyl-CoAs can then enter the β-oxidation pathway to be broken down to form ATP. Studies that have been conducted with hemodialysis (HD) patients suggest that this population has the potential for low concentrations of plasma and tissue free-carnitine

and/or altered carnitine metabolism (2;13). These low concentrations may lead to defects in the way patients on HD metabolize long chain fatty acids, and possibly are associated with some of the previously mentioned symptoms, such as fatigue, muscle cramps, poor muscle strength, poor appetite, and decreased quality of life. Supplementation of patients on hemodialysis has been shown to increase plasma carnitine concentrations.

It may be that low plasma carnitine, especially acylcarnitine, concentrations are associated with poor clinical outcomes in certain patients on hemodialysis. Clinical parameters that are associated with anemia, muscle cramping, quality of life, and nutrition were targeted. By establishing these associations with carnitine, a sub-group of patients, we hypothesized had increased risk for low carnitine values and as well as poor clinical outcomes can be targeted for intervention. The purpose of this study was to determine if correlations exist between various parameters of plasma carnitine and specifically defined clinical and demographic data. In particular, the following questions were addressed: 1) Are there associations between low plasma carnitine concentrations and the following variables: average erythropoietin dose/month, average mannitol dose/month, hematocrit, triglyceride, parathyroid hormone (PTH) and blood urea nitrogen (BUN) concentrations, exercise tolerance, SF36 score, dietary protein intake and kilocalorie, years on HD, treatment time (minutes/hours), co-morbidities (presence of diabetes, left arterial dilation and left ventricular hypertension), medications, age and weight status? 2) What is the mean acyl-to-free plasma carnitine ratio in this hemodialysis patient population? 3) Is there a correlation between acyl-to-free plasma carnitine ratio and the clinical parameters noted above?

Methods

This study was conducted at the Dialysis Center of Lincoln (DCL) in Lincoln, Nebraska. Required approvals for conducting this study were obtained from the Medical Director and the Administrator at DCL and the University Committee on Research Involving Human Subjects (UCRIHS) at Michigan State University.

Sample

The population from which the sample was taken included patients $\geq$ 18 years of age who had been receiving a minimum of four hours of bicarbonate hemodialysis treatments, three times per week for at least one year. Exclusion criteria were: current or previous treatment (within the last two months) with L-carnitine, a severe blood loss, disease affecting skeletal muscle function, severe liver disease and pregnancy. Patients with severe blood loss were excluded because they are typically given increased doses of erythropoietin and iron supplements to help increase blood volume. Patients who have diseases affecting skeletal muscle function were excluded because the disease may confound the impact of carnitine on the skeletal muscle fibers. Additionally, patients with severe liver disease and/or pregnancy may have altered carnitine metabolism for reasons other than uremia. Patients included in this study signed a consent form.

Procedures

Each patient, who signed a consent form, had their blood drawn by a Registered Nurse (RN) or the phlebotomist at DCL on a Wednesday or Thursday of the first week of the month in accordance with the patient's routine monthly blood draw. No patient had been

without dialysis prior to the blood draw longer than 48 hours. A 1 ml aliquot of plasma from the blood sample was collected in a separator tube and frozen at –80° C until it could be analyzed. The plasma was analyzed for free, short and long chain acylcarnitine using the radioenzymatic assay as outlined by Brass and Hoppel (14). Total carnitine was calculated as the sum of the free, short, and long chain acylcarnitine values.

The remainder of the laboratory data were extracted from the routine monthly laboratory profile. These variables were analyzed by Spectra laboratories (Fremont, California). Chronological age at the time of blood draw, the average erythropoietin dose/month and the average mannitol dose/month, a current medication list, the patient's most recent history and physical, the rate of perceived exertion and the perceived dyspnea, and the patient's most recent dialysis prescription were also obtained. The data on left ventricular hypertrophy (LVH) and left arterial dilation (LAD) were obtained from the medical chart based on findings from previous echocardiography. Not all patients had this information. Therefore, data from only a subset of patients were analyzed for LVH and LAD.

On the day of data collection patients who biked during their dialysis treatment were considered "exercisers". The rate of perceived exertion and perceived dyspnea were used to define exercise tolerance (15). Both of these scales use a 1 to 20 rating with 20 as the most exertion or dyspnea and 1 the least. The patients who bike, do so almost every dialysis session. Each time a patient rides the bicycle, the patient's rate of perceived exertion and perceived dyspnea five minutes into the biking session were recorded into the computer system.

Measurement of patients' perceived quality of life is an important outcome assessment tool. One of the better known tools, the Medical Outcomes Study Short-Form-36 (SF36), has been shown by Kutner and others to have good reliability and validity (16;17). Sloan et al (18) demonstrated an increase in the physical and general well being components of the SF36 after oral supplementation with 1g L-carnitine for six months. The SF36 has eight different scales related to self-perception of health-related quality of life. The range for each of the eight scales is 0 to 100.

The two components examined during our study were the physical and mental components. The quality of life assessment tool, SF36, was administered to the patients at DCL every six months by social workers. The forms were scored by the social workers and the most recent results of the SF36 for the patients who were participating in this study were used. If an SF36 had not been done in the past six months, it was done on the day of blood draw by one of the social workers at DCL. During the week of the blood draw, the dietitians at DCL and the investigator conducted two dietary recalls (24 hour and usual day) on each patient participating in this study. The completed recalls were analyzed for average kcal and protein content. The analysis of the dietary recalls was done using the software program Nutritionist V produced by N^2 Computing, 1999.

Analysis of Data

Descriptive statistics were done on both the independent and the dependent variables. The continuous independent variables were assessed for associations with the dependent

variables: total, free and acyl (short chain acyl + long chain acyl) plasma carnitine concentrations using the Pearson's Correlation Coefficient. However, when the variables were skewed, Spearman's Correlation Coefficient was used. To better describe, the patients they were separated based on gender, mannitol use, presence of diabetes (type 1 and 2) and the presence of LVH. T-test analyses were done for the binary variables to determine associations with the dependent variables and the continuous independent variables. T-test analyses were also used to compare mean protein and energy intake between patients on and not on mannitol. To determine whether any of the independent variables were predictors (when controlling for potential confounders) for the dependent variables, multiple linear regression was used. Since the purpose of this study was to identify important variables for the next phase of the project, a prospective, randomized, controlled carnitine supplementation study, correlations between outcome variables typically used in dialysis care and carnitine concentrations were deemed significant using a p value of 0.05, and Bonferroni adjustments were not done.

Results

A total of 49 patients met the inclusion criteria. Patient data are described in table 4.1. The mean plasma total, free and acylcarnitine concentrations were 40.3 $\pm$ 11.8 μM/L (SD), 22.8$\pm$ 7.3 μM/L and 17.5 $\pm$ 5.9μM/L respectively. The acyl group is comprised of the short chain and long chain acylcarnitines, the means of which were 10.27 $\pm$ 4.8μM/L and 7.07$\pm$2.7μM/L, respectively. The mean ratio of acyl–to-free plasma carnitine was 0.80 $\pm$ 0.27. The mean and standard deviation for the continuous variables are found in

table 4.2. Significant correlations between carnitine and the independent variables are shown in tables 4.3 and 4.4. Secondary disease states such as: type 1 diabetes and left ventricular hypertrophy (LVH) were trending toward statistical significance with free carnitine. Kilocalorie intake was not significantly correlated with plasma total, free, short chain, or long chain acylcarnitine.

To determine which sub-groups of patients were most at risk for low plasma carnitine concentrations, the patients were separated by gender, type of diabetes, and presence of LVH. Males, but not females, had a positive correlation between the plasma A/F ratio and SF36 physical component (SF36p) as seen in figure 4.1 ($r= 0.54$). When the subjects were separated into those with type 1 diabetes ($n=6$) and those without it, there was a negative correlation between age and the A/F ratio ($r= -0.83$) in subjects with type 1 diabetes and strong negative correlations between age and free carnitine and total carnitine in patients with LVH.

To examine the relationships between plasma carnitine, mannitol use, kcal/protein intake and intradialytic weight gain the following analyses were done. First, a one-way anova was used to compare mean protein and kcal intake, mean normalized protein catabolic rate and mean intradialytic weight gain at the time of blood draw between patients on mannitol and those who were not on mannitol. No significant difference was found between the means for any of the variables. The second analysis completed was to select those patients receiving mannitol and conduct Pearson's correlation coefficient analyses between kcal and protein intake and the carnitine moieties. Significant correlations were

found between protein intake and both plasma long chain acylcarnitine ($r=0.57$, $p<0.05$) and free carnitine ($r=0.48$, $p<0.05$) concentrations. There were also trending correlations between kcal intake ($r=0.39$, $p<0.05$) and long chain acylcarnitine and significant correlations between kcal intake and free carnitine ($r=0.51$, $p<0.05$).

When Multiple Linear Regression Models were applied to the independent variables, the models found in Table 4.5 significantly predicted Total, Free and Long Chain Acylcarnitine but not Short Chain Acylcarnitine.

Discussion

The hemodialysis population is getting older and is tending to have more co-morbidities; by establishing these associations with carnitine, a sub-group of patients who is at increased risk for low carnitine values and thereby poor clinical outcomes can be targeted for intervention. Carnitine intervention in these subgroups may improve the patients' quality of life and overall mortality.

In healthy adult subjects the mean acyl-to-free plasma carnitine ratio is 0.26. (13). In pediatric patients, the only population for which established abnormal cut points have been specified, an acyl-to-free plasma carnitine ratio ≥ 0.4 is used to define carnitine deficiency (19). The mean acyl- to-free plasma carnitine ratio in this study was 0.8 ± 0.27.

All of the patients had acyl-to-free carnitine ratios above the defined pediatric level of carnitine deficiency.

In our study, the acyl-to-free plasma carnitine ratio was associated with duration of treatment, triglyceride and serum parathyroid hormone. When just males were examined, the ratio was positively correlated with the physical component of the quality of life indicator, short-form-36 and in patients with type 1 diabetes the ratio was positively correlated to age. It is unclear why males and not females have this association. However, it may be connected to the fact that males may perceive quality of life to being more closely tied with physical ability than females.

Currently acyl-to-free plasma carnitine ratios have not been examined in many studies. However, in 1992 Golper et al.(20) reported that HD dialysis patients had acyl-to-free plasma carnitine ratios between 0.6 and 0.9. Debska-Slizien et al.(21) in an observational study involving 37 hemodialysis patients and 29 controls found increased acyl-to-free plasma ratios when compared to the control group, and in the hemodialysis group a positive correlation was seen between acyl-to-free plasma carnitine ratio and high-density lipoproteins. Alhomida (22) studied the effect of hemodialysis on peripheral serum lymphocyte concentrations and plasma carnitine. When he compared healthy controls to 27 HD patients he found no difference in the lymphocyte concentrations between the two groups, but they did find the acyl-to-free plasma carnitine to be higher in hemodialysis patients when compared to the controls ($p<0.01$). When Kopple et al (8) conducted a study with 8 hemodialysis patients, they looked at the carnitine content in the muscle and found muscle acyl-to-free carnitine concentrations were similar to healthy controls.

Neither Kopple et al (8), Golper and Ahmad (23), nor Alhomida (24) reported correlations between any clinical variables and the acyl-to-free plasma carnitine ratio.

Labonia et al (25), Nikolas et al (26), Caruso et al (1), and Trovato et al (27) have all addressed the relationship between plasma carnitine concentrations and red blood cells. Labonia (28) studied the effect L-carnitine had on the red blood cell sodium transport mechanism. These researchers found a decrease in the sodium-potassium pump activity in uremic, end-stage renal disease patients, ($n=8$) compared to controls ($p<0.05$). After supplementation with L-carnitine the sodium-potassium pump activity increased. The authors of this study stated that in uremic patients, there existed sodium-potassium pump inhibitors, possibly long chain fatty acids. The L-carnitine supplementation could increase fatty acid transport and oxidation thereby decreasing the concentration of the sodium-potassium pump inhibitors. The improved pump activity could stabilize the red blood cell membrane and increase the half-life of the cell. Labonia et al (29) as well as Nikoloas et al, (30) Caruso et al (1) and Trovato et al (31) found increases in either hematocrit or hemoglobin with the supplementation of L-carnitine. In our study, we also found a positive correlation between hematocrit and free carnitine. However, when this data was subjected to the multi-variate analysis no correlations were found. Additionally, we did not find any correlations between erythropoietin amounts and carnitine concentrations.

Serum parathyroid hormone (PTH) positively correlated with total, free and acyl-to-free plasma carnitine concentrations. In 1987, Smogorzewski et al. (32) demonstrated that

chronic renal failure is associated with impaired oxidation of long chain fatty acids by skeletal muscle possibly due the availability of carnitine or to an effect on the enzyme, carnitine palmitoyl transferase, responsible for the formation of the long chain fatty acid-carnitine complex. In a later study, by the same laboratory (33) the researchers felt a connection between PTH and chronic renal failure existed and was due to elevated PTH concentrations causing increased calcium ions in the skeletal muscle. These high concentrations of calcium were postulated to be interfering with the action of the carnitine palmitoyl transferase and thus affecting long chain fatty acid oxidation. In our study the correlation between serum PTH and the acyl-to-carnitine ratio was positive. As PTH concentrations increased, so did the acyl-to-free carnitine ratios, possibly indicating that higher PTH concentrations negatively affect the metabolism of acylcarnitine. Bellingheri et al. (34) showed decreases in muscle cramping and interdialytic fluid gains in 14 dialysis patients after supplementation with oral carnitine. Mannitol, (a drug used to decrease muscle cramping while on dialysis) was used as a clinical outcome to represent the muscle cramping. When we examined patients on mannitol the drug used for muscle cramping due to rapid fluid removal on hemodialysis, we found higher plasma total, free and acylcarnitine concentrations in patients using mannitol ($p<0.05$). We then examined the relationship with carnitine and protein and found a positive correlation between protein intake and plasma long chain acylcarnitine concentrations. This relationship is logical because carnitine is found in high protein foods. Blood urea nitrogen concentrations were also positively correlated with free and total carnitine concentrations. Nitrogen and carnitine are both found in high protein foods and this may account in part for the concurrent increase in blood urea nitrogen and carnitine concentrations. It is

possible that those patients who have higher consumption of food (including high protein/carnitine foods) are also consuming more fluids and in turn must have more fluid removed.

Recently, Elisaf et al. (5) showed a decrease in the mean triglyceride concentration of 28 HD patients who had received intravenous carnitine supplementation ($p<0.05$), again possibly decreasing their risk for cardiovascular accidents. We found a positive correlation between serum triglyceride concentrations and the acyl-to-free plasma carnitine ratio ($p<0.05$), but not with free or total carnitine. Correlations between left ventricular hypertrophy (suggestive of hypertension) and left arterial dilation (suggestive of dilated cardiomyopathy) were found in our study. These results are not clear since the pathophysiology of these two disease states are basically opposed. Furthermore, it is difficult to ascertain correlations such as these with retrospective left ventricle hypertrophy data obtained via chart reviews because of the inconsistent nature of this data. These data can only direct future research to an area that needs further clarification from research involving more precise methods of determining left ventricular hypertrophy and its association with carnitine.

One of the secondary roles of carnitine is the release of mitochondrial coenzyme A (CoA) from acyl-CoA when the free CoA supply becomes limited due to activation and accumulation of metabolites in the mitochondrion (35). Medications such as aspirin can affect the free CoA supply and therefore result in the release of mitochondrial CoA by carnitine. Patients on hemodialysis take aspirin because of its anti-clotting properties

during the dialysis process and for underlying heart disease. It was hypothesized that patients taking medications that ultimately affect CoA concentrations could have an impact on plasma carnitine concentrations due to the increased need for CoA to metabolize these medications. Subsequently, we did find lower concentrations of total carnitine in patients taking aspirin ($p<0.05$).

Similar to the findings by Caruso et al (1) we found a positive correlation between age and total and free plasma carnitine concentrations. As patients' age they lose muscle mass and 97% of the carnitine in the body is stored in the skeletal muscle. Therefore, as they lose muscle, they may also lose carnitine stores.

In summary, this study has shown that HD patients have elevated acyl-to-free ratios and these ratios correlate with the physical component of the SF36 in males and age in type 1 diabetic patients. Furthermore, certain patients, such as older, females, and those on aspirin therapy or those with low dietary protein intake may be at greater risk for lower concentrations in plasma carnitine status. Finally, plasma free and total carnitine status appears to be further compromised as age increases; and with increases in BUN, Hct and PTH. These results suggest certain subgroups of patients may need to be targeted for further studies with carnitine replacement therapy, i.e. long-term patients, older patients, patients with LVH and left arterial enlargement, females, and patients on aspirin therapy. Therapy could be targeted to these patients and outcomes monitored. In addition, the SF36 assessment may be a useful outcome monitor in males and type 1 diabetics. The

data from this study warrant more in-depth investigation of the role of carnitine in this vulnerable patient group.

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Table 4.1

Demographic Data

Age (mean±SD)	60±16
Males	48%
Mannitol use	46%
Erythropoietin use	88%
Patients who biked during dialysis	46%
Patient on lipid lowering medications	25%
Aspirin use	35%
**Type 1 Diabetes Mellitus	15%
**Types 2 Diabetes Mellitus	29%
**Left Ventricular Hypertrophy	25%
**Congestive Heart Failure	15%

*from a subset of patients (n=43) with this data available
**per a retrospective chart review

Table 4.2

Mean and Standard Deviations for Continuous Clinical Parameters

Continuous Variable	Mean±SD*
Serum Blood Urea Nitrogen	51 ±20 mg/dl
Serum Parathyroid Hormone	238 ± 188 pg/ml
Serum Hematocrit	36±4
Left Arterial Dilation (n=12)	4.2±0.6
Ejection Fraction % (n=14)	51±15.2
Monthly ave Epogen dose	1100 ± 3631 units
Monthly ave Mannitol dose	15.2±5.3
Serum Triglyceride	169 ±107 mg/dl
Respiratory Perceived Exertion (n=17)	10±3
Perceived Dyspnea (n=17)	0.8± 1.3
Kilocalorie	1516 ± 563
Protein	72 ± 30 g
Duration on Dialysis	45 ± 34 m
Treatment Time	3.95 ± 0.73 hr

* Data are presented as mean ± SD for n=49, unless otherwise reported

Table 4.3

Continuous Independent Variables Significantly Associated with Carnitine*

Independent Variable	Total Carnitine	Free Carnitine	Long Chain Acyl Carnitine
Age	r = -0.32	r = -0.41	r =-0.41
Dietary Protein Intake			r=0.33
Serum Blood Urea Nitrogen	r = 0.29	r = 0.41	r=0.50
Serum Parathyroid Hormone	*r = 0.33	*r = 0.36	**r=0.34
Serum Hematocrit		r = 0.30	
Left Arterial Dilation	r = -0.67	r = -0.62	r=-0.80
Ejection Fraction %	r = 0.55		r=0.52

* Pearson's Correlation Coefficient, $p < 0.05$

** Spearman's Correlation Coefficient, $p < 0.05$

Table 4.4

Categorical Independent Variable Significantly Associated with Carnitine

Independent Variable	Total Carnitine	Free Carnitine
Gender		
Female	37.9±2.08	21.0±1.29*
Male	43.1±2.73	25.0±1.60
Aspirin		
Yes	37.4±2.61**	21.2±1.59
No	42.5±2.20	24.0±1.35
Mannitol		
Yes	45.3±2.56*	25.3±1.54*
No	36.1±2.00	20.6±1.28

Data are presented as mean ± SE for n=49
* = $p<0.05$, ** = $p=0.08$

Table 4.5

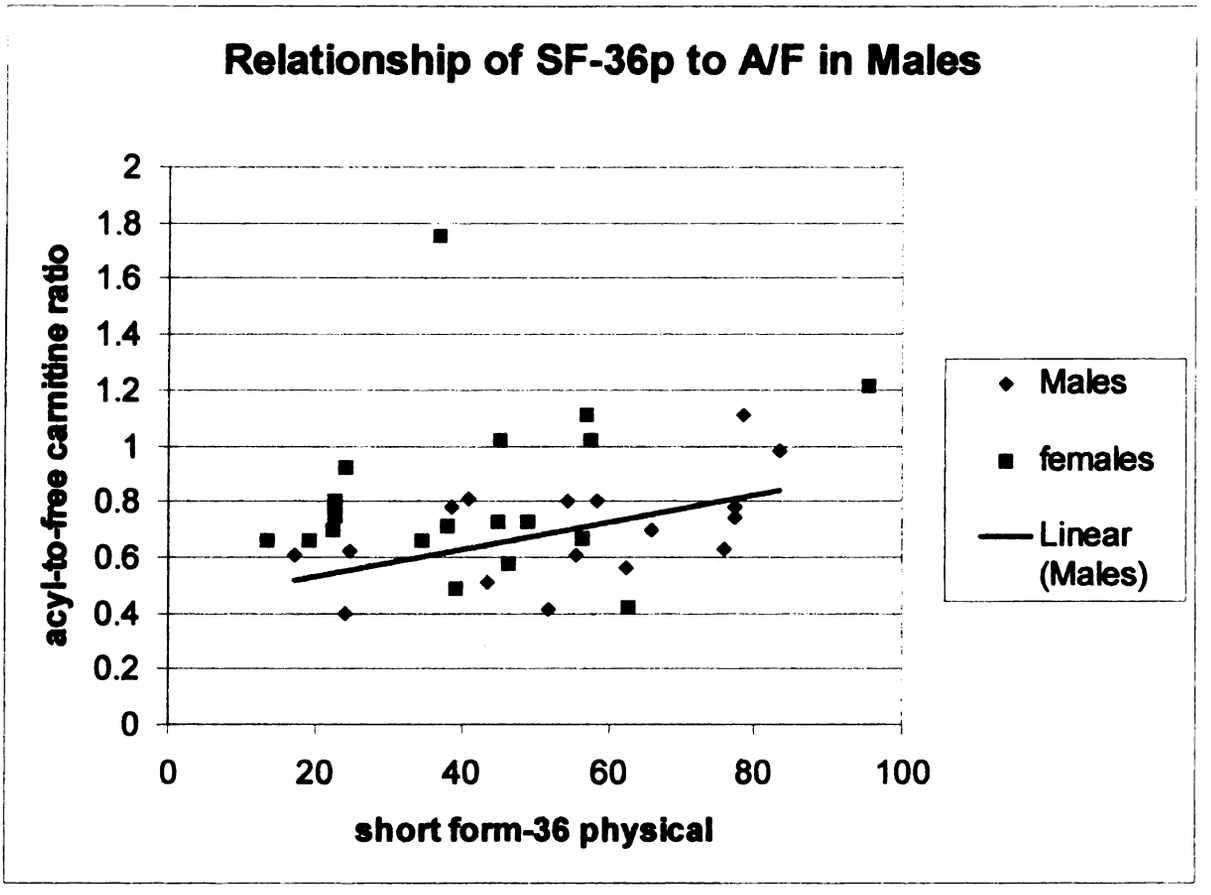
Multivariate Linear Regression Models Predicting Total, Free, and Long Chain Acylcarnitine Values

	Model 1*	**Model 2****	**Model 3*****
Predictive Variables	(β-Coefficients)	(β-Coefficients)	(β-Coefficients)
Blood Urea Nitrogen (mg/dl)	0.412	0.437	0.469
Age (years)	- 0.185	- 0.249	- 0.330
Weight (kg)	0.176	0.204	- 0.116
Parathyroid Hormone (pg/mL)	0.253	0.203	0.034
Gender	- 0.161	- 0.157	- 0.276
Protein Intake (g)	- 0.008	0.061	0.097
Pre-Albumin (mg/dL)	- 0.105	- 0.039	- 0.284

*R^2= 0.44, $p<0.05$ predicting total carnitine
** R^2= 0.55, $p<0.05$ predicting free carnitine
** R^2= 0.62, $p<0.05$ predicting long-chain acylcarnitine

Figure 4.1

Relationship of Short Form-36 Physical Composite Score to Plasma Acyl-to-Free Carnitine Ratio in Male Hemodialysis Patients



*r=0.54, $p<0.05$

Chapter 5

Phase II, Aim 2

Carnitine Treatment Improved Quality of Life Measures in a Sample of Midwestern Hemodialysis Patients

Alison L. Steiber, Alan T. Davis, Leslie Spry, Jennifer Strong, Gina Miller, Mary Lou Buss, Michelle M. Ratkiewicz, Lorraine J. Weatherspoon

Abstract

Previously we demonstrated that selected groups of hemodialysis (HD) patients might be more likely to have abnormalities of carnitine metabolism. The purpose of the present study was to examine the effects of carnitine therapy in these selected groups of HD patients on quality of life measures and erythropoietin dose. This was a double-blind, randomized controlled trial, in which 50 HD patients were treated with either 2 g IV carnitine or placebo. The treatment period was for 24 weeks. Thirty-four patients (15 in the treatment group) completed the study. The mean age was 69±15 yrs, 35% were female and 44% had diabetes. Mean initial plasma total, free, short chain acyl and long chain acyl carnitine concentrations (μmol/L; mean±SEM) were 35.9±1.8, 18.2±1.1, 11.6±0.6 and 6.0±0.3, while the plasma acyl-to-free carnitine ratio was 1.02±0.05. With respect to the Medical Outcomes Short Form-36 (SF36), improvements from baseline were noted in the treatment group (n = 13) for role-physical (33.9±1.9 to 43.2±3.0, $p<0.05$) and the SF36 physical component summary score (36.1±2.7 to 39.7±2.3, $p=0.09$), relative to changes in the control group (n = 14). The erythropoietin dose over the 24 week period was reduced from baseline in the treatment group, relative to the placebo group (-1.62±0.91 versus 1.33±0.79 units erythropoietin dry weight^{-1}

hemoglobin concentration^{-1}, respectively, $p<0.05$). After 24 weeks of IV carnitine therapy, SF36 scores were improved and erythropoietin doses were reduced in HD patients, relative to the control group.

Introduction

L-carnitine is a compound found in high protein foods and is produced by the liver, kidney, and brain. The metabolic functions of carnitine are to transport fatty acyl-CoA into the mitochondrial matrix, export products of β-oxidation from peroxisomes, and release mitochondrial CoA from acyl-CoA when free CoA supplies are limited. Research has demonstrated altered free and acylcarnitine concentrations in patients receiving hemodialysis treatment (1-3).

Recently, L-carnitine treatment was approved by the Centers for Medicare & Medicaid Services (CMS) for national reimbursement in hemodialysis patients with either erythropoietin resistance or chronic hypotensive episodes during dialysis treatment. Following the establishment of the CMS reimbursement policy, a carnitine consensus group performed a thorough review of the existing literature, and determined that there were four main types of symptoms that may accompany altered carnitine metabolism in dialysis patients: anemia hyporesponsive to synthetic erythropoietin, intradialytic hypotension, cardiomyopathy and skeletal muscle weakness (4). The current title for this set of symptoms is "dialysis-related carnitine disorder" (DCD).

The mechanism for DCD has yet to be determined and in fact is probably multi-factorial. Research has been done to suggest that the alteration of carnitine may occur prior to

dialysis being initiated (5;6) and therefore the alteration may be due, in part, to dietary intake or uremia itself. High protein foods, containing higher amounts of L-carnitine, are often restricted in the diets of patients with renal insufficiency to preserve kidney function and decrease uremic side effects. Uremia itself has been shown to alter fatty acid (7) and amino acid (8) composition in patients. Therefore, uremia may alter the type of carnitine (free or acylated), the utilization, or the function of carnitine as well. Furthermore, as the kidney fails, the synthesis of carnitine may decrease.

Previous work in this lab has shown that a subset of hemodialysis (HD) patients may be at greater risk for plasma carnitine alterations (1). This group of high-risk hemodialysis patients has lower plasma free carnitine concentrations, but normal total carnitine concentrations. Administration of L-carnitine as a therapeutic agent in this group of patients increases plasma free carnitine concentrations and by mass action leads to removal of long and medium chain acylcarnitine moieties. The acylcarnitines will leave the cell and be removed from the plasma by dialysis.

In an effort to show that L-carnitine treatment can benefit all dialysis patients, the general hemodialysis population has been the target for the majority of the previously reported carnitine research. However, Elisaf et al (9) found there were certain patients who responded to levocarnitine and other patients who did not. In contrast to theories from previous studies, the purpose of this study was to select a particular section of the hemodialysis population, determined to be at increased risk for altered carnitine metabolism by the criteria established previously (1), and monitor these patients for

benefits from L-carnitine treatment, including perceived quality of life and selected clinical outcomes.

Subjects and Methods

Subjects

This study was conducted at a Midwestern dialysis center. Required approvals for conducting this study were obtained from the Medical Director and the Administrator at the Dialysis Center of Lincoln, the Community Institutional Review Board in Lincoln, NE, and the University Committee on Research Involving Human Subjects at Michigan State University. All procedures followed were in accordance with the ethical standards of Michigan State University' Committee on Research Involving Human Subjects.

The population from which the sample was taken included patients $\geq$ 18 years of age, who had been receiving a minimum of three hours of bicarbonate, low acetate hemodialysis treatments, three times per week and who had been receiving dialysis for at least one year. In addition, those patients admitted into the study met two or more risk factors for a compromised serum carnitine as established in our previous study (1). These risk factors were 65 years of age or older, at least one year duration of treatment, female, use of aspirin and/or mannitol, and the presence of type II diabetes mellitus, left atrial dilation and/or left ventricular hypertrophy. Exclusion criteria were current or previous treatment (within the last two months) with L-carnitine, a severe blood loss, disease affecting skeletal muscle function, severe liver disease, pregnancy and/or free carnitine > 40μmol/L.

Patients with severe blood loss were excluded because they are typically given increased doses of Epogen® and iron supplements to help increase blood volume. Patients who

have diseases affecting skeletal muscle function were excluded because the disease may confound the impact of carnitine on the skeletal muscle fibers. Patients with severe liver disease and pregnancy may have altered carnitine metabolism for reasons other than uremia. Additionally, in order to comply with the Federal Drug Administration's criteria for supplementation with intravenous levocarnitine in the hemodialysis population, all patients with a plasma free carnitine concentration greater than 40 μmol/L were excluded. Patients included into this study signed a consent form and were randomized into control and treatment groups.

Procedures

This was a prospective, randomized, double blind, clinical research study performed between September, 2001 and March, 2002. The variables and their times of collection are outlined in table II. The patients had their blood drawn for carnitine analysis during the study on the first week of the baseline month and at 12 and 24 weeks, in accordance with routine monthly blood draws. The blood sample was collected in a heparin tube, from which the plasma was obtained and stored at -80° C until analysis. Plasma carnitine concentrations were analyzed as described previously (10;11).

The mean erythropoietin dose was recorded monthly during the course of the study. The erythropoietin administration data were analyzed as the change from baseline to post-treatment. Erythropoietin usage was analyzed using an erythropoietin resistance index, created by dividing the patients' pre- and post-treatment erythropoietin doses by the patients' dry weight and their hemoglobin concentration.

To determine changes in Kcal and protein consumption, dietary recalls were collected using the multiple pass method (12), a 24 hour recall and a typical day recall. These values were averaged in order to account for individual variations. Nutritionist V produced by N^2 Computing (First Databank, Inc., San Bruno, CA) was used for nutrient analysis. Normalized Protein Catabolic Rate (nPCR) was calculated using the following formula:

PCR = 0.22 + 0.036 X interdialytic rise in BUN * 24/Interdialytic interval in hours

To normalize the PCR: nPCR = PCR/weight (kg)

The seven point SGA form (13) and mid-arm muscle circumference (MAMC) (14) were used to assess nutrition status at baseline, 12 weeks, and 24 weeks. SGA is a subjective tool used to assess nutritional status and does not require laboratory values. The tool combines a series of dietary, gastrointestinal, clinical, and functional status questions in combination with a brief physical exam to assess nutritional status. MAMC was used as a surrogate marker of lean body mass.

The Medical Outcomes Short Form-36 (SF36) surveys were administered and scored by the social worker at the dialysis centers (15) at baseline and at 24 weeks. The SF36 is a perceived quality of life assessment, which has eight domains used to comprise either a mental (MCS) or a physical (PCS) composite score. Each domain was scored on a scale of 0 to 100, with 100 being the highest perception of functioning. The domains are physical functioning, role-physical, bodily pain, general health, mental health, role-

emotional, social functioning, and vitality. The MCS and PCS were normalized as described by Ware and Kosinski (16). Further data were collected from medical records and random data audits were conducted to ensure consistency and accuracy of collection.

Treatment

Those patients who signed an informed consent and met basic inclusion criteria were screened for selected "high carnitine risk" criteria (Table I). The purpose for the high carnitine risk criteria was to get a sample of hemodialysis patients most likely to have altered carnitine metabolism. The criteria used has was taken from previous studies conducted with carnitine (1). The patients must have met a minimum of two of the criteria in table I to participate in the study. Those patients eligible to participate in the study were randomized into either the treatment or the control group by a co-investigator who did not participate in the data collection process. This investigator and the pharmacist were the only persons not blinded. Those patients who were in the treatment group received 20mg/kg Carnitor® (Sigma-Tau Pharmaceuticals, 800 South Frederick, Gaithersburg, MD 20877) actual body weight intravenously, post hemodialysis treatment, three times per week intravenously. The carnitine was administered to the patients in an infusion of 100 ml of normal saline over 10 minutes by the dialysis center nursing staff. The patients in the control group received an intravenous placebo of 100 ml of normal saline over 10 minutes. The amount of carnitine needed was calculated using the patients' dry weight from the baseline of the study. By diluting the carnitine in normal saline and giving it in an infusion, the potential odor of the drug was masked and blindness maintained. The treatment period was for 24 weeks.

Analysis of Data

Data were analyzed using SPSS version 11.0 (SPSS Inc., Chicago, IL). For all of the quantitative variables except carnitine, the difference in the amount of change between the control and treatment groups was determined using the analysis of covariance, using the baseline measurement as the covariate. The carnitine data for the baseline, three month, and six month periods were analyzed using a repeated measures analysis of variance. Differences in descriptive variables were determined using either the t-test for quantitative variables, or the χ^2 test for nominal variables. Associations were analyzed using Pearson's correlation coefficient. The variables were analyzed for multivariate relationships using multiple regression. Significance was assessed at $p < 0.05$.

Results

Of the 50 study patients, two were removed due to plasma free carnitine concentrations > 40μmol/L, seven voluntarily withdrew (one prior to baseline collection), and seven died from causes unrelated to carnitine. Of the 34 remaining patients (15 treatment, 19 placebo), seven declined to complete the final SF36 survey. However, data for the other variables were collected on these seven patients. For the variable SF36, there were 13 in the treatment group and 14 in the placebo group.

Table 5.1 outlines the baseline descriptive data for the study patients. The primary etiologies for the patients on hemodialysis were diabetes and hypertension. At baseline there were significant differences between treatment and placebo groups for the variable nPCR, while the remaining baseline descriptive and primary outcome variables were not significantly different between the two groups. There were no statistically significant differences at baseline for any of the carnitine variables between the two treatment groups (Table 5.2). There were numerous correlations between the carnitine fractions at baseline and some of the descriptive parameters (Table 5.3). When the independent predictors were analyzed using multiple regression to determine predictors for the three month MAMC measurements, baseline plasma free carnitine and baseline BMI were significant ($r = 0.83$).

The primary outcome variable for this study was perceived quality of life questionnaire, SF36. There were a statistically significant improvement in the carnitine group for role physical (Table 5.4), with a trend towards significance noted for the PCS ($p = 0.09$),

relative to the control group. In addition, there were no statistically significant differences for the baseline SF-36 values between the treatment and control groups. The significance level for the baseline difference in role physical score was 0.053. However, when the baseline measurements of the role physical score were used as the covariate in the ANCOVA, they did not achieve statistical significance.

There was a significant difference in the change in utilization of erythropoietin in the carnitine treated group, relative to the controls. Relative to baseline, at six months the carnitine group showed a decrease of 1.62±0.91 units erythropoietin dry weight^{-1} [Hb]$^{-1}$, relative to an increase of 1.33±0.79 units erythropoietin dry weight^{-1} [Hb]$^{-1}$ in the control group. Of the other variables analyzed for changes between pre- and post-treatment with carnitine (dietary intake, nPCR, SGA, MAMC, BUN, triacylglycerols, hemoglobin, hematocrit), none demonstrated statistically significant changes between the treatment and the control group.

Discussion

The main endpoints for this study were the Medical Outcomes Short Form-36 domains and the physical composite score. Within the treatment group, a significant increase was seen in the SF36 domain role physical, relative to controls. Furthermore, the physical composite score was trending strongly toward significance. This was particularly striking given the small sample size and the high attrition rate experienced in this study.

The SF36 is a self-reported assessment tool designed to measure the patient's health related quality of life. It has been well validated in the hemodialysis population by Kutner

(17;18) and others (19;20). As a group, the baseline mean composite scores (prior to normalization) found in our study were similar to the ones documented by Curtin, Lowrie and DeOreo (20) for the physical composite score but slightly higher than the reported mental composite score.

Other interventions, such as exercise, have been reported to increase the SF36 scores of end stage renal disease patients (21). However, previous studies with levocarnitine treatment have been unable to demonstrate increases in the physical and mental composite score of the SF36. Sloan et al (3) reported findings from a randomized clinical trial where the levocarnitine was given orally for six months. In the Sloan study, the SF36 was given every one-and–a-half months, after the first one-and-a-half month period, an improvement could be seen in the physical functioning and general health domains. However, this effect seemed to disappear by the sixth month. Baseline descriptive data were not reported for the Sloan study, therefore it is difficult to compare the two groups. However, it may have been the route of administration of the levocarnitine that led to the discrepancy between the two studies. Oral levocarnitine is only partially absorbed and a bacteria-mediated process degrades the unabsorbed portion (22;23). The by-products of this degradation process, trimethylamine and γ-butrobetaine, may be toxic and are excreted by the kidney. Patients with end stage renal disease are not able to excrete these by-products. Therefore, it is possible the by-products increase in concentration toxic level (24).

The Chief trial (2) was recently conducted using quality of life as one of its main end-points. In this trial, patients were given intravenous levocarnitine for six months and the

Kidney Disease Questionnaire (KDQ) was the assessment tool used to measure perceived quality of life. The KDQ is validated for measuring quality of life in patients with end stage renal disease, and it was assessed at baseline, midpoint and at the end of the trial (2). The subjects in this trial were younger than in this trial (mean age 42-48 verses 67 years), the Chief trial treatment groups had fewer patients with diabetes (11-25% verses 45%), and their mean nPCR was higher (1.02-1.17 verses 0.9). At the end of the Chief trial, the researchers did not find an improvement in the total score of the KDQ. However, they did find a significant improvement in the fatigue portion of the tool.

The results found in this study may have been in part to the way the patients were selected or the elderly, co-morbid status of the patients. Other factors that may have played a role are the patient's familiarity with the SF36 and the people administering it.

The difference in the change of the erythropoietin doses between the treatment and placebo groups is not unexpected. Other researchers such as Caruso et al (25) and Kletzmayr et al (26) have reported improvements in erythropoietin dosing with levocarnitine treatment. Caruso et al. specifically reported this finding in hemodialysis patients greater than 65 years of age (25), whereas Kletzmayr et al found anemia and erythropoietin resistance index responders and nonresponders. In the Kletzmayr et al study those patients who responded had significantly reduced erythropoietin resistance indexes (26). About 50% of the treatment group in this study were responders to levocarnitine treatment.

Although nutritional status was not affected by levocarnitine treatment, it did seem to be associated with baseline plasma carnitine status. The independent variables that were correlated with baseline carnitine were for the most part nutritional assessment indicators (nPCR, serum BUN, albumin, dietary protein intake, and SGA). All of the correlations were positive, indicating the better the nutritional status, the higher the baseline plasma carnitine concentrations.

Furthermore, baseline plasma carnitine was associated with 12-week BMI and MAMC values and as can be seen by the multiple linear regression model, plasma free carnitine is predictive, along with BMI, for the 12-week MAMC. Plasma free carnitine may be an early indicator of lean body mass atrophy. Plasma free and acyl carnitine are lost in the dialysis process. The body stores (97% of which are in the muscle) may be partly responsible for restoring plasma carnitine concentrations, especially when the diet is low in levocarnitine sources (red meats, poultry, and dairy).

The limitations of this study were low subject numbers and high attrition due to death, voluntary withdrawal, and refusal to participate. The study was done solely by the volunteer time and effort of the patients, nurses, social workers and dietitians at the dialysis unit. Therefore, missing data did occur and patients were less likely to complete the quality of life questionnaire. Strengths of the study included the knowledge of the social workers with the SF36, patient randomization with a placebo group and double blinding the participants throughout the study period.

In conclusion, this study was unique due to the specific selection criteria of the participants and the dietary data collected. Quality of life was significantly improved in the treatment group of this hemodialysis patient sample in the area of the domain role-physical, with a trend towards improvement in the physical composite score. Furthermore, mean erythropoietin dose changes between the groups were significantly different, with the treatment group dose decreasing and the placebo group increasing. Significant associations were found between the dependent variables (total, free, short chain acyl and long chain acylcarnitine) and many nutrition related independent variables. Future studies could focus on further narrowing the selection criteria and more detailed investigation of the relationship between quality of life and physical functioning with levocarnitine treatment.

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Table 5.1

Characteristics of the study groups at the start of the study[1]

	Placebo Group (n = 19)	Carnitine Group (n = 15)
Age (y)	69.4±3.4	67.6±3.9
Women (%)	21.1%	53.3%
Etiology of Disease		
Diabetes (%)	47.3%	40.0%
Hypertension (%)	21.1%	26.7%
Other (%)	31.6%	33.3%
Duration of treatment (y)	4.0±0.5	3.8±0.6
BMI (kg/m^2)	28.6±1.4	30.4±1.8
MAMC (%)	22.5±1.0	22.5±2.0
Dietary Intake Energy (kcal/d)	1194±96	1374±99
Dietary Intake Protein (g/d)	47.5±4.6	54.8±5.5
nPCR[2]	0.78±0.03	0.92±0.05
BUN	39.9±2.3	45.1±3.7
Albumin (g/dl)	3.8±0.1	3.7±0.1
Triacylglycerol (mg/dl)	173.4±26.4	180.4±19.0
HDL	41.6±2.7	38.1±4.1
Hemoglobin (g/dl)	11.5±0.3	11.3±0.4
Hematocrit (%)	36.3±0.9	36.9±1.2

[1] All quantitative data are expressed as mean±SEM. BMI, Body mass index; MAMC, Midarm muscle circumference; nPCR, normalized protein catabolic rate; BUN, blood urea nitrogen; HDL, high density lipoprotein.

[2] Significantly different at $p < 0.05$, using the unpaired t-test.

Table 5.2

Plasma carnitine concentrations by treatment group[1]

	Control Group			Carnitine Group		
	Baseline (n = 24)	12 weeks (n = 22)	24 weeks (n = 18)	Baseline (n = 25)	12 weeks (n = 20)	24 weeks (n = 14)
Total Carnitine (μmol/L)	35.7±2.0	34.4±3.1	34.2±3.2	39.1±2.9	184.1±27.2	334.8±41.1
Free Carnitine (μmol/L)	19.0±1.4	18.7±1.8	15.1±1.4	20.6±1.8	125.7±18.2	177.9±17.8
SCAC (μmol/L)	10.8±0.6	11.3±1.3	12.6±1.8	11.7±1.0	54.5±9.7	90.7±7.9
LCAC (μmol/L)	5.8±0.3	4.4±0.4	6.50±1.15	6.7±0.5	27.3±5.0	66.2±22.6
Acyl/Free Carnitine Ratio	0.96±0.08	0.90±0.07	1.50±0.26	0.94±0.06	0.70±0.06	0.89±0.10

[1] All data are expressed as mean±SEM. SCAC, short chain acylcarnitine. LCAC, long chain acylcarnitine. All variables had significant effects for treatment, duration of treatment, and the interaction between treatment and duration of treatment, using a repeated measures analysis of variance and significance assessed at $p < 0.05$.

Table 5.3

Associations between descriptive variables and various baseline carnitine fractions.[1]

	Free Carnitine	SCAC	LCAC	Total Carnitine
Baseline				
nPCR	0.39		0.35	0.38
Serum BUN	0.60	0.37	0.47	0.59
Serum [Albumin]		0.41		
Protein Intake	0.38			0.32
Serum Triacylglycerol		0.35		
Serum HDL			0.40	
3 Months				
MAMC	0.50		0.39	0.49
BMI			0.31	

[1] All values represent Pearson's r, and are significant at $p < 0.05$. SCAC, short chain acylcarnitine; LCAC, long chain acylcarnitine; nPCR, normalized protein catabolic rate; BUN, blood urea nitrogen; HDL, high density lipoprotein; MAMC, Midarm muscle circumference; BMI, Body mass index.

Table 5.4

Short Form 36 (SF36) scores at baseline and at six months for the control and carnitine groups.[1]

	Control (n = 14)		Carnitine (n = 13)	
	Baseline	6 Months	Baseline	6 Months
Physical Function	30.3±3.0	29.2±3.3	37.0±2.3	35.9±2.5
Role Physical[2]	40.6±2.6	40.1±3.0	33.9±1.9	43.2±3.0
Bodily Pain	50.2±2.4	47.9±3.9	42.9±4.3	50.9±3.7
General Health	40.3±3.5	39.2±3.4	42.9±1.9	43.2±2.3
Vitality	46.3±4.0	48.0±3.3	44.1±2.6	46.1±2.4
Social Function	41.8±3.3	46.4±3.2	44.7±3.5	50.5±2.7
Role Emotion	43.3±2.9	47.0±2.8	42.4±3.8	50.5±2.6
Mental Health	49.5±3.7	46.2±4.4	50.2±3.4	51.4±2.7
PCS[3]	37.3±2.7	35.7±3.2	36.1±2.7	39.7±2.3
MCS	49.6±3.5	51.8±2.8	49.7±3.7	54.2±2.2

[1] All data are expressed as mean±SEM. PCS, physical component summary measure; MCS, mental component summary measure. All scores were normalized to the 1998 general United States population, as described (16).

[2] The change from baseline in the carnitine group was significantly greater than the change from baseline in the control group, using ANCOVA, at $p < 0.05$.

[3] The change from baseline in the carnitine group showed a trend for a greater increase from baseline than the change from baseline in the control group, using ANCOVA, at $p = 0.09$.

Chapter 6

Phase II, Aim 3

The impact of intravenous carnitine treatment over 24 weeks on the concentration and distribution of acylcarnitines in hemodialysis patients

Steiber AL, Weatherspoon LJ, Hoppel CL, Minkler, P, Spry L, Davis AT

Abstract

The purpose of this study is to examine the effect of carnitine supplementation, on acylcarnitine (AC) detected in plasma in a hemodialysis (HD) population at risk for compromised carnitine status. The study was a double blind, randomized trial conducted over 24 weeks. Fifty patients met the previously defined inclusion and exclusion criteria and were randomized into either a control (IV normal saline) or a treatment (IV L-carnitine at 20 mg/kg) group. Blood collection occurred at randomization, and weeks 12, and 24. Total carnitine (TC) and free carnitine (FC) were analyzed using radioenzymatic assay; and AC moieties were analyzed using HPLC/mass spectrometry. At 24 weeks, 32 completed the study. Mean age was 68±14 yrs, 38% were female, 45% had diabetes.

All p<0.05	Total Carnitine	Free Carnitine	Short Chain Acylcarnitine	Long Chain Acylcarnitine sum	Ayclcarnitine to Free Carnitine ratio	acetylcarnitine/other Acylcarnitine
Treatment	334±154	178±67	90±30	0.7±0.3	0.9±0.4	8.5±1.5
Control	34±13	15±6	13±8	0.3±0.2	1.5±1.1	4.9±1.5

With carnitine treatment FC and AC increased. In the treatment group the magnitude of the change in AC depended on the acyl moiety. The largest increased was in C2 with 700 fold. Medium acyls increased intermediately the greatest amount C10/3x, C8/5x, C6/2x and C4/6x. These data indicate an increased metabolism of LCACs in hemodialysis patients.

Introduction

In chronic kidney disease patients receiving hemodialysis (CKDH), carnitine metabolism has been of interest because of its potential effects on muscle fatigue and quality of life. Patients with CKDH frequently are affected by secondary conditions such as anemia, myopathy, hyperparathyroidism, left ventricular hypertrophy, hypertension and osteoporosis. Many CKDH patients have altered plasma carnitine concentrations and altered acyl-to-free ratios (1-3). When compared with healthy subjects, patients with CKDH are reported to have higher acylcarnitine concentrations and lower plasma free carnitine concentrations; whereas, the total carnitine concentrations remain within the normal range (1).

In healthy subjects, the most prevalent form of plasma acylcarnitine is acetylcarnitine. Following carnitine removal, coenzyme-A (Co-A) is attached to acetate and acetyl Co-A is formed. Acetyl Co-A is a substrate for many metabolic pathways and in a healthy population is the primary metabolite resulting from β-oxidation. However, in plasma of CKDH patients, increased amounts of medium and long chain acylcarnitine concentrations are reported in the literature (1;4;5). The increase in medium and long chain acylcarnitine moieties may be due to an alteration in β-oxidation and/or a decrease

in excretion of these metabolites. Long chain fatty acyls have deleterious effects on cellular membranes and metabolic functions. Hypothesized mechanisms for the negative effects are 1. interference with sodium-potassium transporters in cell membranes (6) and 2. detergent effects on cellular phospholipid membranes (7).

It is possible that elevation of an individual acylcarnitine or a group of acylcarnitine moieties can be identified as a marker for altered carnitine metabolism. Additionally, increased concentrations of acylcarnitine moieties may have negative effects on clinical parameters associated with carnitine metabolism. Clinical parameters associated with carnitine concentrations are dietary protein, serum albumin, serum blood urea nitrogen, serum hemoglobin and hematocrit, presence of diabetes mellitus, or left ventricular hypertrophy (1;8;9).

Treatment with L-carnitine (biologically active form) may effectively drive long chain acylcarnitine moieties through β-oxidation by mass action, thus decreasing mitochondrial acylcarnitine concentrations and increasing plasma acylcarnitine concentrations. As with many dysfunctions, not all patients have the same degree of alteration. Therefore, to be able to detect a true treatment effect, a sample of patients with significant carnitine metabolism alteration, would be necessary. We previously demonstrated that patients with some defined carnitine risk facotrs had serum carnitine abnormalities (1). Therefore, the purpose of this study was to: 1) identify acyl carnitine moieties present in the plasma of a group of hemodialysis patients at risk for abnormal carnitine status and 2) determine if treatment with L-carnitine alters acylcarnitine moieties in the patients.

Methods

This study is one portion of a larger randomized, double-blind, placebo controlled clinical trial. The study was conducted at a midwestern dialysis center. Required approvals for conducting this study were obtained from the Medical Director and the Administrator at the Dialysis Center, the Community Institutional Review Board, and the University Committee on Research Involving Human Subjects at Michigan State University. All procedures followed were in accordance with the ethical standards of the Committee on Research Involving Human Subjects and all patients signed an informed consent prior to participating the in the study.

Subjects

This data was obtained from a randomized, double-blind, clinical trial. The patients were enrolled in the clinical trial based on the following inclusion criteria: $\geq$ 18 years of age, receiving a minimum of three hours of bicarbonate, low acetate hemodialysis treatments, three times per week, for at least one year. Additionally, patients admitted into the study had two or more risk factors for a compromised carnitine status: $\geq$ 65 years old, 1 year duration of treatment, female, use of aspirin and/or mannitol, or presence of type 2 diabetes mellitus, left atrial dilation and/or left ventricular hypertrophy.

Exclusion criteria were: current or previous treatment (within the last two months) with L-carnitine, a severe blood loss, disease affecting skeletal muscle function, severe liver disease, pregnant, and free carnitine > 40μmol/L. Patients with severe blood loss were excluded because they are typically given increased doses of synthetic erythropoietin and

iron supplements to help increase blood volume. Patients who have diseases affecting skeletal muscle function were excluded because the disease may confound the impact of carnitine on the skeletal muscle fibers. Patients with severe liver disease and pregnancy may have altered carnitine metabolism for reasons other than uremia. Additionally, in order to comply with the Federal Drug Administration's criteria for supplementing with intravenous levocarnitine in the hemodialysis population, all patients with a plasma free carnitine concentration > 40 were excluded.

Procedures

Patients had their blood drawn for carnitine analysis on 0, 12 and 24 weeks, in accordance with routine monthly blood collection. Plasma from the blood sample was spun and collected in a heparin tube, frozen to -80°C and shipped with dry ice to Spectrum Laboratory in Grand Rapids, MI. Radioenzymatic assay was used to determine concentrations of total carnitine, free carnitine, short chain acylcarnitine, long chain acylcarnitine, and the plasma acyl-to-free carnitine ratio (10). In Grand Rapids, when the samples were thawed for analysis a portion was separated and re-frozen for shipment to Louis Stokes Veterans Administration Medical Center in Cleveland, OH where analysis of acylcarnitines using high performance liquid chromatography with mass spectrometry (HPLC/MS/MS) was conducted (11).

In addition to the plasma samples, descriptive and nutritional parameters were collected from routine laboratory reports, medical records, and patient interviews. During patient interviews, the 7-point version of Subjective Global Assessment (SGA) (12;13), two

multiple pass method 24 hour recalls(14), and a Medical Outcomes Short Form-36 (SF36) (15;16) were conducted.

The SGA tool was used to rank the presence of malnutrition using diet, gastrointestinal, clinical, and functional status questions in combination with a brief physical exam to assess nutritional status. Dietary intake of kilocalories and protein were measured with two 24 hour recalls preformed for a typical dialysis and non-dialysis day with the multiple pass method (14). The two day intake data were averaged (kcal/day or protein g/day), then calculated to kcal/kg/day and protein g/kg/day. The SF36 is a tool used to measure perceived quality of life. It has eight domains, which were scored from 0-100, from these eight domains; two composite scores (physical and mental) are calculated by a weighted mean (17).

Treatment with L-Carnitine

During the clinical trial, patients randomized to the treatment group, received 20mg/kg Carnitor® actual body weight, post hemodialysis treatment, three times per week intravenously. L-carnitine was administered to the patients in an infusion of 100ml of normal saline over 10 minutes by the dialysis center nursing staff. Patients randomized to the placebo group received an intravenous placebo of 100 ml normal saline over 10 minutes. The amount of carnitine administered was calculated using the patients' dry weight from baseline. By diluting the carnitine in normal saline and giving it in an infusion, the potential odor of the drug was masked and blindness maintained. The treatment period was for 24 weeks.

Analysis of Data

Data were analyzed using SPSS version, 11.0, 2001. Descriptive, correlation analysis, and one-way ANOVA analyses were performed to detect associations, mean differences, and changes from pre- and post-intervention periods. Significance was defined at $p<0.05$.

Results

Fifty patients were recruited for the study. Two were removed from the analyses due to high plasma free carnitine (>40 μmol/L), seven voluntarily withdrew from the study (one prior to baseline data collection), and seven died during the study. When baseline characteristics where compared between patients who completed the 24 week trial and those who did not, there were no perceivable differences. Descriptive characteristics at 0, 12 and 24 weeks are reported in Table 6.1. The patients had the following primary etiologies 36% diabetes mellitus type 2, 28% hypertension, 9 % diabetes mellitus type 1, 6% obstructive uropathy and 21% other etiologies. Additional descriptive can be found in Chapter 5 (Steiber et al. submitted for publication).

Baseline plasma total, free, short and long chain acylcarnitine concentrations for all the patients (n=47) and for healthy fasted controls (n=30) are shown in table 6.2. Baseline plasma acylcarnitine concentrations for healthy, fasted controls (n=29) and all study participants with a baseline free carnitine < 40 μmol/L are found in table 6.3. After recruitment, patients were randomized into treatment (n= 23) and placebo (n=24) groups. There were no mean differences in acylcarnitine moiety concentrations between the

placebo, the healthy control or the treatment groups at baseline. The mean plasma total, free, short, and long chain acylcarnitine concentrations from each group are found in figure 6.1. Figure 6.2 shows the linear increase of plasma free carnitine throughout the 24 week study.

During the 24 week treatment period, the acylcarnitine moieties within the placebo group remained stable. The treatment group has significantly higher values in all acyl moieties at 12 and 24 weeks when compared with the placebo group, except isovaleryl, which never reached statistical significance. In addition, 2-methylbutynyl reached a statistical significance only at week 24. Figure 6.3 is a chromatogram of a treatment group patient's acylcarnitine moieties from baseline to 12 and 24 weeks. Figures 6.4a-d depict mean acylcarnitine changes which occurred within the treatment group from baseline to 12 and 24 weeks. Measuring the change in the ratio of plasma acetylcarnitine to measurable sum of propionyl to 9,12-octadecadienyl is also an indicator of change due to L-carnitine treatment and this can be found in Figure 6.5.

Baseline statistical analyses with acylcarnitine concentrations reveal correlations with laboratory values, anthropometric measures, and dietary factors table 6.4. Additionally, the overall nutritional status assessment tool, subjective global assessment, was positively correlated with decanylcarnitine ($r=0.30$, $p<0.05$), laurylcarnitine ($r=0.32$, $p<0.05$), and palmitylcarnitine ($r=0.45$, $p<0.01$).

Within the treatment group, the magnitude of change in acylcarnitine depended on the acyl length. The largest increase was a 700 fold increase in acetylcarnitine. Medium acylcarnitines increased intermediately, with the largest increases in butynylcarnitine (6 fold), octanylcarnitine (5 fold), decanylcarnitine (3 fold), and hexanylcarnitine (2 fold).

Discussion

The data presented in this paper are the first to be reported in the medical literature regarding plasma acylcarnitine moieties in a defined sample of pre- and post-treatment hemodialysis patients. At baseline the mean free, short, and long-chain acyl lengths were outside normal parameters (10) and parameters established by Borum (18). Plasma free carnitine was on average 50% less than normal values; whereas plasma short and long chain acylcarnitine concentrations were approximately double normal values.

At baseline the mean concentration of acetylcarnitine for the whole sample was not significantly different than the healthy, fasted controls. Acetylcarnitine is the primary substrate for many metabolic pathways. However when carnitine metabolism is altered theoretically, acetylcarnitine concentrations would decrease. Recently, Hoppel et al (submitted for publication) reported significantly lower concentrations of acetylcarnitine within their dialysis sample. These patients differed from the ones in our study by many factors. Primarily they were not selected for risk of altered carnitine metabolism which was the case with our patients. Additionally, the patients in the study by Hoppel (2005) were and they were younger, had greater ethnic diversity, and a lower frequency of

diabetes (2). It is unclear why this younger, potentially healthier sample had lower acetylcarnitine concentrations than our older sample.

When examining the results for acylcarnitine concentrations greater than acetylcarnitine, between the dialysis sample and the healthy, fasted, controls, the data may appear to be conflicting. In table 6.2 dialysis patients' values are reported as significantly different than the healthy, fasted control values. This finding contradicts table 6.3 which reports dialysis acylcarnitine moieties are not significantly different than the healthy, fasted controls. The difference occurs because HPLC/MS/MS in this analysis measured 19 acylcarnitine moieties. However, over 39 acylcarnitine moieties exist. Therefore, when the radioenzymatic assay measured short and long chain acylcarnitine, it captured all the acyl moieties, not just those detected in the HPLC/MS/MS analysis. The acylcarnitine moieties not captured by HPLC/MS/MS are the ones elevated, hence the discrepancy between high concentrations found by radioenzymatic assay and normal concentrations by HPLC/MS/MS.

As anticipated, following treatment with L-carnitine, acylcarnitine moieties, except for isovaleryl- and 2-methylbutyrylcarnitine, were significantly higher in the treatment group than in the placebo group. Additionally, 24 week acylcarnitine concentrations were higher than baseline concentrations within the treatment group. However, within the treatment group, when 12 week acylcarnitine concentrations where compared to 24 week acylcarnitine concentrations, a significant difference was not found. Pharmacologically, homeostasis is found when the treatment drug reaches a stable concentration in the

homeostasis was not reached at 12 weeks but may have been reached by 24 weeks. Conversely, Evans et al (19), suggested that steady-state was reached by 9 weeks in a pharmacokinetic trial conducted on 12 hemodialysis patients when free and acetylcarnitine were analyzed. In our study, although the free carnitine continued to rise throughout the 24 weeks of treatment by week 12 the acylcarnitine concentrations did stabilize for some patients.

The variables associated with acylcarnitine concentrations were all surrogates for nutrition status and nutritional intake (dietary protein intake, weight, MAMC, SGA, nPCR, BUN, serum albumin, hematocrit and SF36, general health domain). This is not surprising, in previous work we and others have found correlations with total, free and acylcarnitine and nutrition parameters (1;8;20). Additionally, serum albumin is bound to long chain fatty acyls for transport, therefore it is not surprising that the concentrations of long chain fatty acids correlate positively with serum albumin. It is possible that classes of acylcarnitine moieties may be used as indicators for clinical or metabolic alterations that could benefit from carnitine treatment.

The body's predominate source of carnitine is dietary carnitine, lysine and methionine and the predominate storage site is muscle. Currently, national food and nutrient databases, such as the United States Department of Agriculture's Handbook 8, do not contain information on carnitine content within foods; however, meat and dairy sources are believed to have the highest concentrations (21;22). Dietary protein intake was positively correlated with total and free carnitine, but short and long chain acylcarnitine

did not (Steiber et al., manuscript submitted for publication). It is interesting that 2-methylbutyrylcarnitine, but no others, was significantly associated to the actual intake of protein. Whereas, indicators of visceral and somatic protein status such as serum albumin, nPCR and serum BUN are correlated with medium chain acylcarnitine moieties such as: tetradecanyl, dodecanyl, octadecanyl, hexadecanyl (serum albumin); short chain acylcarnitine moieties such as: butynyl (nPCR) and acetyl, propionyl, butynyl, and isobutynyl (serum BUN). The differences in associations may derive from the multiple aspects that affect visceral and protein status which are not due to protein intake. Two of these aspects many be metabolic stress (inflammation, wound healing, fever, uremia) and treatment effect (dialysis, medications). Further investigation with advanced biochemical and anthropometric techniques such as C-reactive protein, lipid peroxidation parameters, and DEXA are warranted to examine these relationships.

In summary, plasma acylcarnitine concentrations in CKD patients receiving hemodialysis are altered. Treatment with L-carnitine does increase total and free carnitine as well as the acylcarnitine moieties. Steady-state of free carnitine does not occur prior to 12 weeks but may occur near 24 weeks. Finally, classes of acylcarnitine concentrations are directly correlated to visceral and somatic nutrition indicators. Future, research will be needed analyze all the possible carnitine moieties and to establish associations between nutritional status and acylcarnitine moieties.

metabolisms. Lipid peroxidation may affect lipid metabolism and thereby acylcarnitine moiety concentrations and finally, DEXA measures lean body store which is the primary location for carnitine storage.

In summary, plasma acylcarnitine concentrations in CKD patients receiving hemodialysis are altered. Treatment with L-carnitine increases total and free carnitine as well as the acylcarnitine moieties. Steady-state of free carnitine does not occur prior to 12 weeks but may occur near 24 weeks. Therefore, outcome measures established to measure the effectiveness of carnitine treatment should be done at a minimum, up through 24 weeks. Finally, classes of acylcarnitine concentrations are directly correlated to visceral and somatic nutrition indicators. Future, research will be needed analyze all the possible carnitine moieties and to establish associations between nutritional status, factors impacting nutrition status, and acylcarnitine moieties.

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Table 6.1

Descriptive Characteristics at Baseline, 12 and 24 Weeks

Variable	Normal Ranges for Dialysis Patients	Baseline n=47	12 Weeks n=41	24 Weeks n=34
Age (years)		68±14	NC	NC
Gender (%female)		38	NC	NC
Body Mass Index	>25=overweight, >30=obese	27.8±6.5	28.3±6.4	27.4±9.6
Dietary Intake (Kilocalories & kilocalories/kg)	30-35 kilocalories/kg > 60years 35 kilocalories < 60years	1277±443 17±6	1336±395	1483±593
Dietary Intake (Protein (g) & g/kg)	1.2	53±26 0.7±0.4	57±20	60±26
Normalized Protein Catabolic Rate	>1.2	0.9±0.2	1.0±0.3	0.9±0.3
Serum Albumin (mg/dL)	>4.0	3.7±0.4	3.7±0.5	3.7±0.4
Serum Triglycerides (mg/dL)	<200	161.7±92.7	172.5±99.4	180.5±108.1
Serum Hemoglobin (g/dL)	11-12	11.4±1.3	11.6±1.3	12.1±1.1

*data shown as mean ±SD

Table 6.2

Baseline Plasma Total, Free, Short, and Long Chain Acylcarnitine Concentrations (μmol/L)

	Whole Sample, Non-fasted* n=47	Healthy, Fasted Controls n=30
Total Carnitine	36±11	46±10
Free Carnitine	19±7	37±8
Short Chain Acylcarnitine	11.1±4.0	5.7±3.5
Long Chain Acylcarnitine	6.0±1.9	3.7±1.5
Acyl-to-Free Ratio	1.0±0.3	0.3±0.2

*radioenzymatic analysis of plasma samples that were shipped to the laboratory on dry ice and frozen to -80°C until analysis (10)

Table 6.3

Baseline Acylcarnitine Concentrations for Total Sample and Healthy, Fasted Controls at Baseline, 12 and 24 Weeks (μmol/L)

Plasma Acylmoiety concentrations	Healthy Controls n=29	Whole Group at Baseline n=47
Acetyl	5.58±2.24	5.32±2.06
Propoinyl	0.27±0.10	0.20±0.10
Butynyl	0.04±0.12	0.08±0.13
Isobutynyl	0.01±0.03	0.08±0.18
Isovaleryl	0.01±0.03	0.00±0.01
2-methylbutynyl	0.00±0.01	0.16±0.49
Hexanyl	0.02±0.05	0.00±0.01
Octanyl	0.10±0.10	0.04±0.10
Decanyl	0.12±0.12	0.03±0.07
Dodecanyl	0.08±0.08	0.04±0.03
Tetradecanyl	0.02±0.01	0.03±0.01
9-hexadecenyl	0.03±0.03	0.02±0.01
Octadecanyl	0.02±0.01	0.02±0.01
9-octadecanyl	0.14±0.06	0.11±0.05
9, 12-octadecadienyl	0.09±0.04	0.06±0.04

*data reported as mean±SD

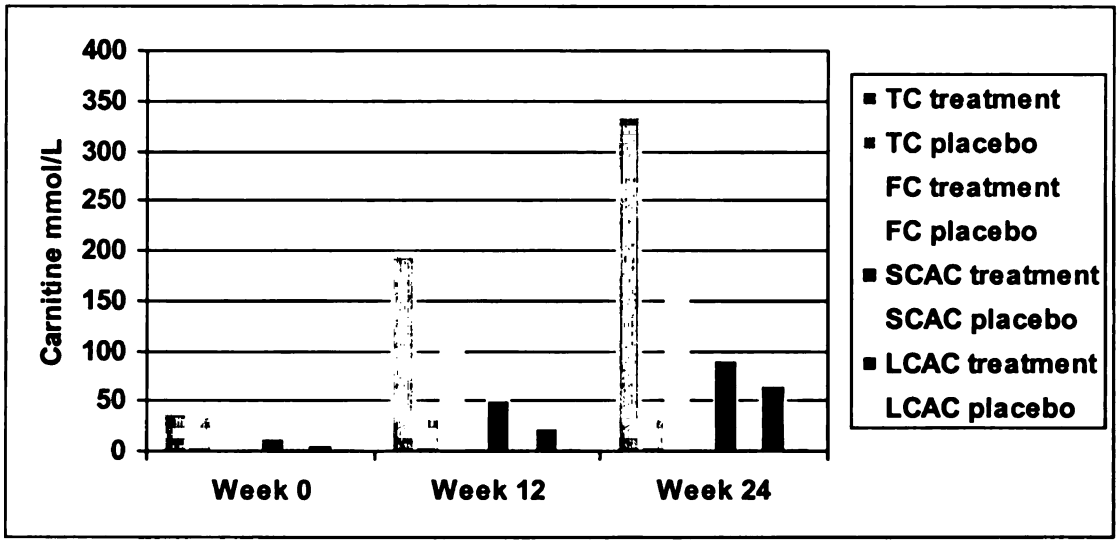
Table 6.2

Associations Between Post-treatment Plasma Acylcarnitine Moieties and Nutritional Parameters

Nutritional Parameter	Acetyl	Propoinyl	Butynyl	Isobutynyl	Isovaleryl	2-methyl-butynyl	Hexanyl	Octanyl	C9	Decanyl	9-hexadecenyl	Tetradecanyl	Octadecanyl
Mid-Arm Muscle Circumference									r=-0.40				
Serum Blood Urea Nitrogen	r=0.42	r=0.68	r=0.79	r=0.44		r=0.73			r = 0.47				
Serum Albumin	r=-0.38						r=-0.54	r=-0.40		r=-0.37			
Protein Catabolic Rate			r=0.40									r = 0.40	
Tricep Skinfold											r=0.38		
Body Mass Index											=0.38		
kilocalorie/kg body weight											r = -0.41		
# of Muscle Cramps					r=0.57							r = 0.40	r = 0.41
Mean erythropoietin dose													r = -0.37

All r values presented are statistically significant, $p<0.05$; n= 34

Figure 6.1. Mean Plasma Carnitine Concentrations



TC – total carnitine, FC – free carnitine, SCAC – short chain acylcarnitine, LCAC- long chain acylcarnitine; Radioenzymatic assay technique, between treatment and placebo groups - $p<0.01$

Figure 6.2. Plasma Free Carnitine at 0, 12 and 24 weeks

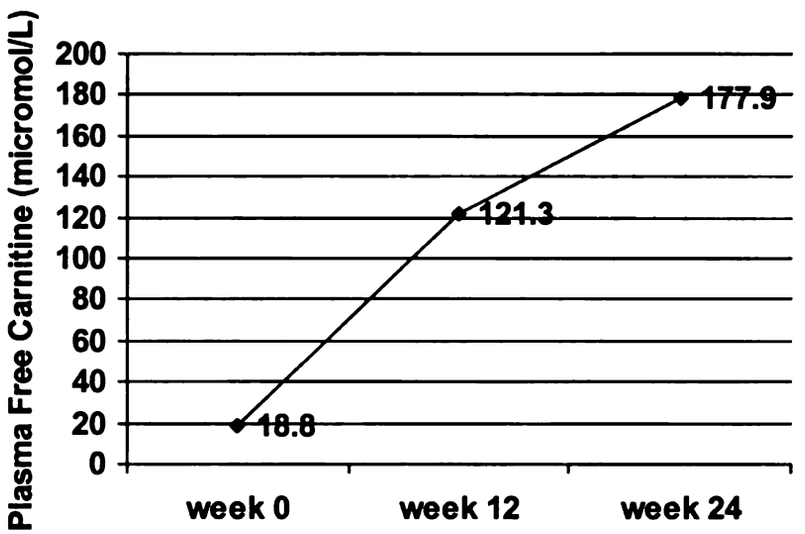


Figure 6.3 Chromatograph of One Patient, Baseline, 12 and 24 weeks

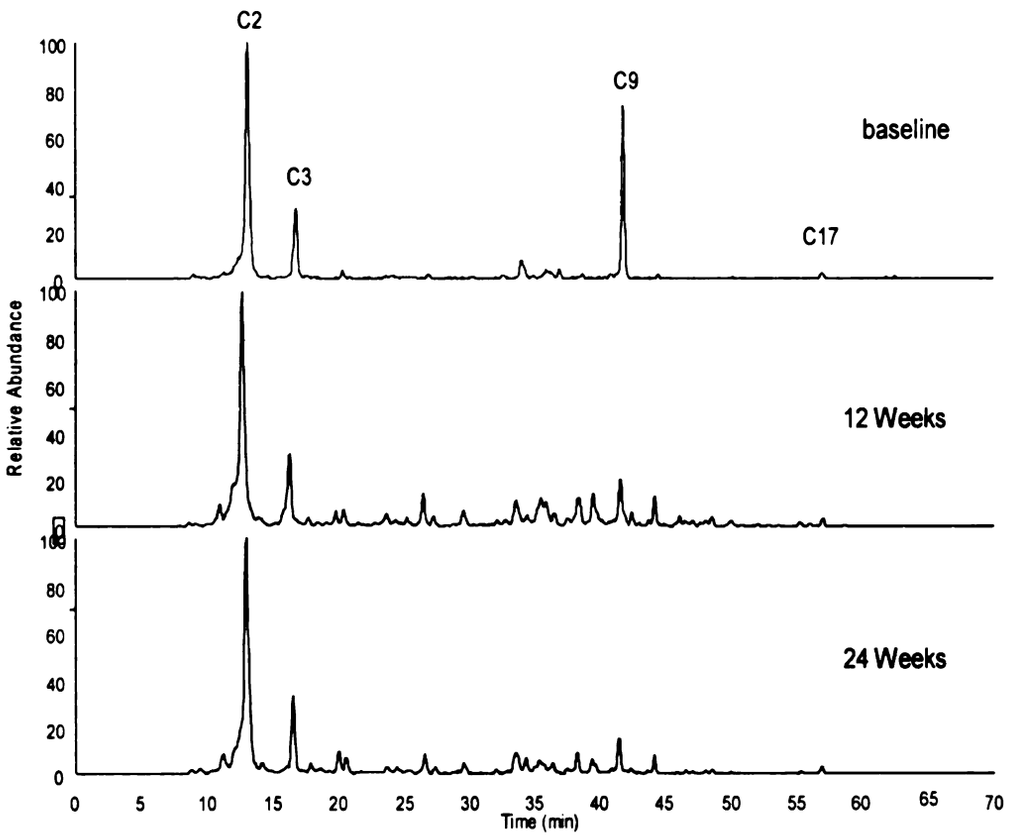
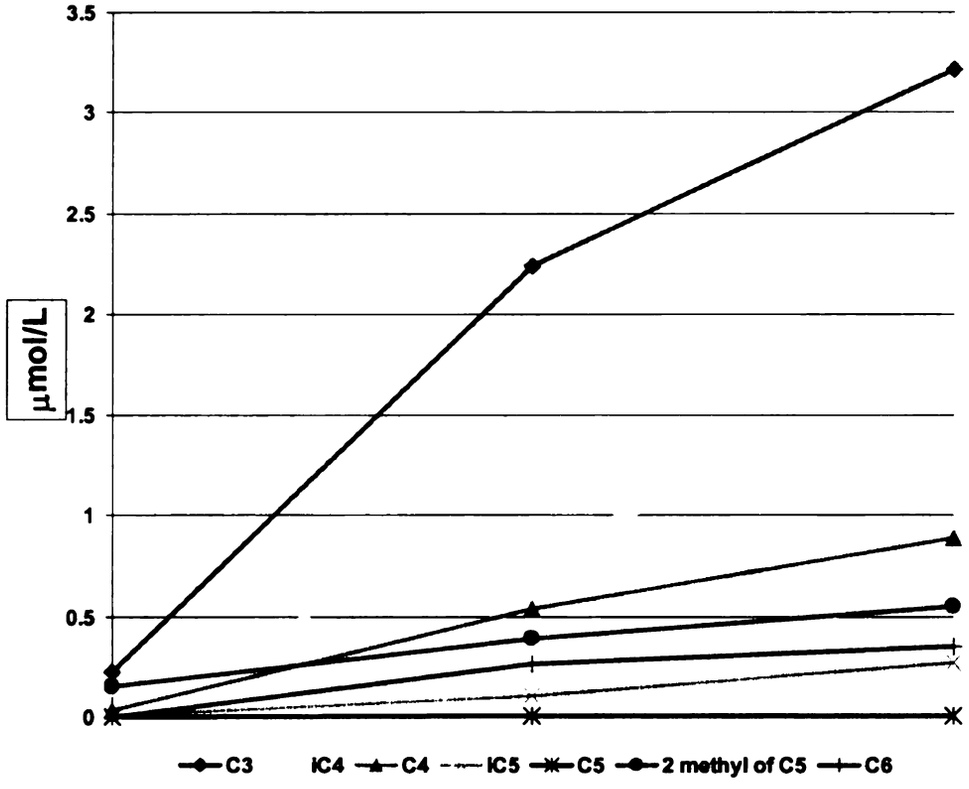
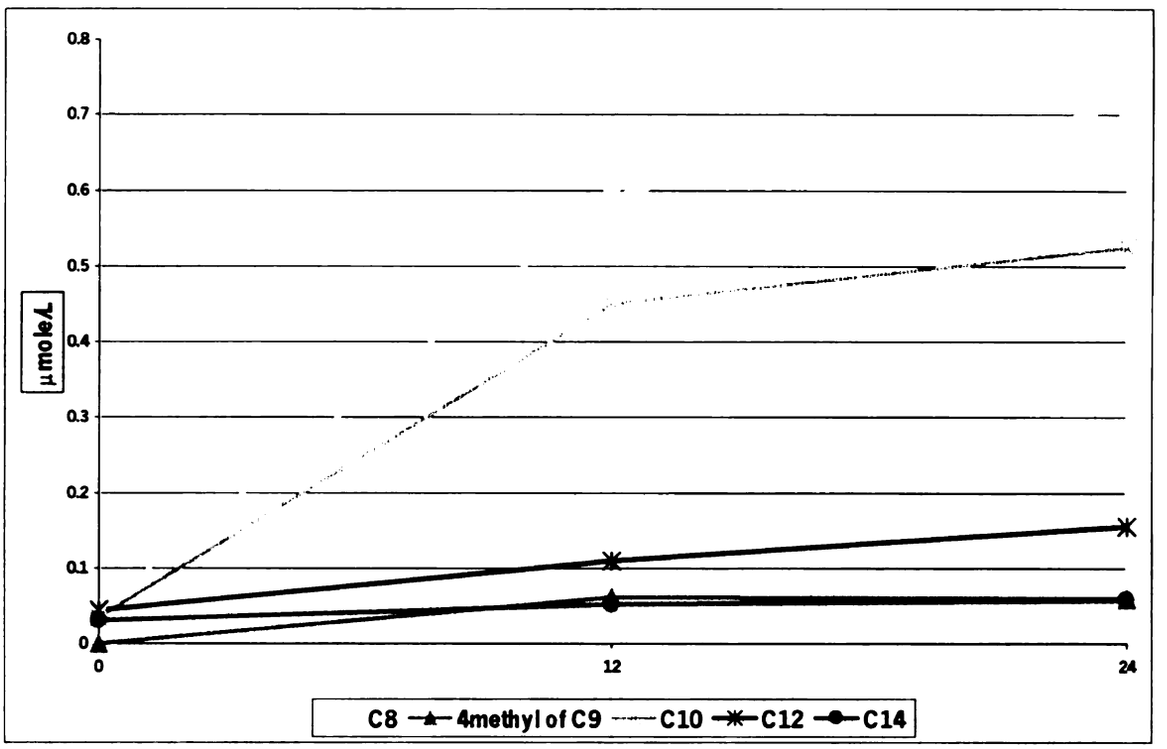


Figure 6.4 Treatment Group Baseline, 12 and 24 weeks of Acetyl- through Hexanylcarnitine



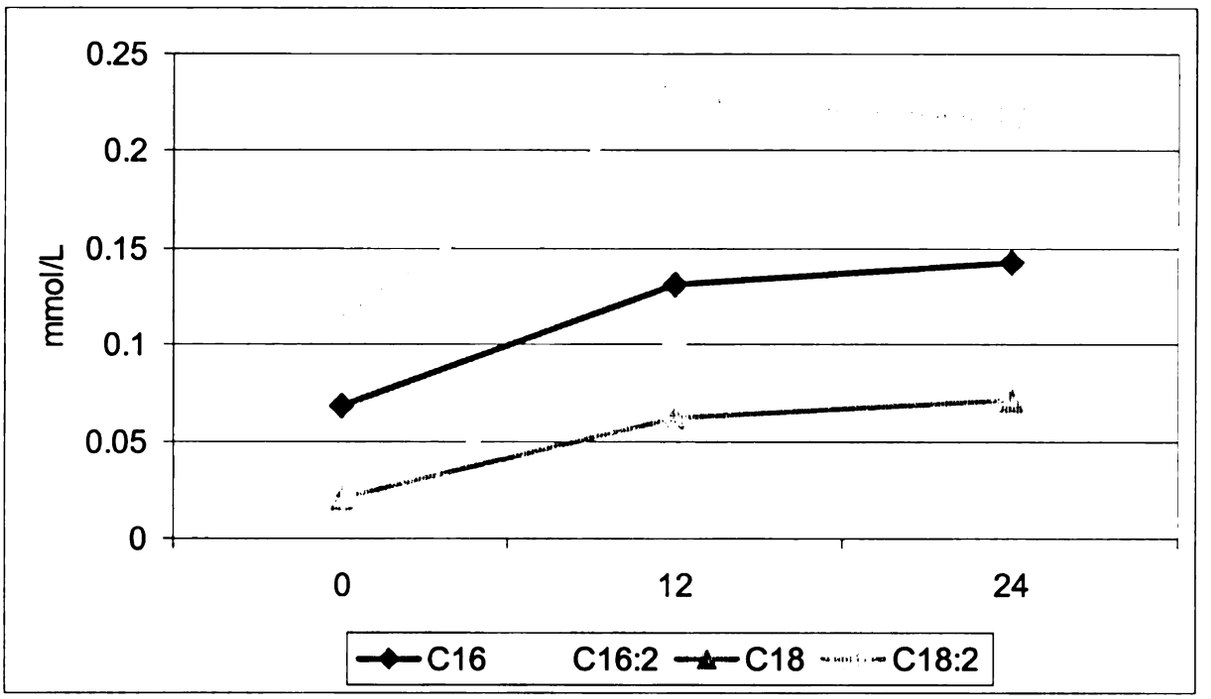
*significance ($p<0.01$) found between baseline and 12, but not between 12 and 24 weeks

Figure 6.5Treatment Group Baseline, 12 and 24 weeks of Octanyl- through Tetradecanylcarnitine



*significance ($p<0.01$) found between baseline and 12, but not between 12 and 24 weeks

Figure 6.6 Treatment Group Baseline, 12 and 24 weeks of Hexadecanyl- through 9, 12-octadecadienylcarnitine



*significance ($p<0.01$) found between baseline and 12, but not between 12 and 24 weeks

Chapter 7

Summary

The prevalence of CKD in the United States is on the rise and with this increase, there are associated increases in healthcare costs, morbidity, and mortality and decreases in quality of life for the individuals affected (1). In patients with CKD, quality of life is determined by multiple factors including adequacy of treatment, the support system, presence of co-morbidities (e.g. diabetes, cardiovascular disease, anemia, muscle weakness) and nutritional status. Validated assessment tools such as the Medical Outcomes Short Form-36 (SF36) can measure perceived quality of life (2). Perceived quality of life has been to be correlated with mortality (3;4).

Carnitine, a low molecular weight molecule found in free and acyl forms, has been shown to be associated with factors affecting anemia and muscular weakness. Approximately 25% of total body in vivo synthesis of carnitine is from lysine, and methionine in a multi-step process and 75% is from dietary sources of carnitine, lysine, and methionine(5;6). Due to its small size and water solubility, carnitine is lost in urine, typically in the acyl form, in healthy humans and lost in the dialysate of CKD patients receiving dialysis treatment. When carnitine is lost during dialysis, it is lost in both the free and acyl forms, thus altering the plasma acyl-to-free carnitine ratio (7).

It has been postulated that in patients with altered carnitine metabolism treatment with L-carnitine moves long and medium chain acylcarnitine moieties out of the mitochondria

and into the plasma where they can be eliminated through urination or in the case of patients receiving dialysis through the dialysis treatment itself. With the removal of acylcarnitine moieties from the mitochondria, metabolic function would return to normal. Normalization of metabolic function could improve clinical parameters such as serum concentrations of hemoglobin, muscle strength, endurance, and prevalence of muscle cramping, thereby improving perceived quality of life.

There are a plethora of studies involving carnitine in the literature, which give clues to the possible role, function, and benefit of carnitine treatment (8-15). A large majority of the treatment studies has been done in the CKD population possibly due to the altered excretion of carnitine in dialysis, the poor intake of dietary carnitine sources, and the prevalence of carnitine deficiency signs and symptoms (muscle weakness and fatigue). However, the CKD literature is rampant with small, poorly controlled studies with conflicting results.

It was hypothesized that not all CKD patients had the same degree of alteration in carnitine metabolism as evidenced by plasma carnitine values nor did they all react the same way to L-carnitine treatment. Therefore, the purpose of this study was to identify risk factors associated with plasma carnitine concentrations, use these risk factors to identify a sample of CKD patients with a high level of altered carnitine metabolism, treat the patient sample with intravenous L-carnitine, and monitor for changes in clinical parameters, perceived quality of life, and plasma acylcarnitine moieties.

To achieve this purpose a two-phase study was conducted using patient interviews and examination, medical records review, and blood collection. Plasma total, free and acylcarnitine concentrations were analyzed with radioenzymatic assay (16) and plasma acylcarnitine concentrations were analyzed using high performance liquid chromatography/mass spectrometry (17). Other variables assessed were descriptive (age, etiology, co-morbidities, treatment and medication information), anthropometric (MAMC, BMI, weight, and height), laboratory (serum BUN, serum albumin, hemoglobin, hematocrit, serum parathyroid hormone, serum triglycerides, and high-density lipoproteins), dietary (energy and protein intake) and perceived quality of life (SF36).

Baseline results for phase I and II indicate that plasma carnitine is correlated with the following parameters: age, dietary protein, nPCR, serum BUN, PTH, hematocrit, triglycerides, high-density lipoproteins, and albumin, left arterial dilation, and ejection fraction percentage. The older patients consuming less dietary protein and thus having lower nPCR, serum BUN, and serum albumin, with higher arterial dilation and lower ejection fraction had lower plasma carnitine concentrations. After 12 weeks of L-carnitine treatment, MAMC and BMI were also positively correlated with plasma carnitine.

At baseline, there were statistically significant differences in plasma carnitine between female and male patients, patients who were and were not using aspirin, and patients who were and were not using mannitol. Females, aspirin users, and non-mannitol users had

lower plasma carnitine concentrations. These correlations and differences in mean values were used to establish risk factors for identifying patients at increased risk for altered carnitine metabolism.

After 24 weeks of treatment with L-carnitine, the following SF36 domains were significantly improved: role-functioning, bodily pain, and role-emotional. The physical composite score was also trending toward significant improvement. A limitation of this data set is the difference at baseline between the treatment and placebo group for the role-functioning variable. This is a very important variable and the most logical for improvement with L-carnitine treatment. The multiple linear regression analysis included group (treatment or placebo), baseline role-functioning and role-functioning change from baseline to 24 weeks demonstrated that when baseline role-functioning, is controlled for, the intervention of L-carnitine is a significant predictor of role-functioning change from baseline to 24 weeks. Patients in the treatment group had significantly improved scores in role-functioning while patients in the placebo group did not change significantly. The findings in the SF36 scores were expected but unique. This is the first study to successfully demonstrate improvement in these variables. Others such as Ahmad et al (9), Sloan et al (10), and Brass et al (15) have attempted to demonstrate improvements with quality of life but have not shown improvements in the SF36 domains.

The second primary outcome of this study was a significant improvement in synthetic erythropoietin doses from baseline to 24 weeks in the treatment group. This is a logical and expected finding. Similar findings have been found in a sample greater than 65 years

of age and in dialysis patients supplemented with iron and intravenous L-carnitine simultaneously (12;18).

The third primary outcome of this study was measurement of plasma acylcarnitine moieties in this high carnitine risk group of patients and subsequent increase of acylcarnitines in the treatment, but not placebo, group. The data from this study indicate that the acetylcarnitine concentrations were within normal limits. This was unexpected given the elderly age and high percentage of patients with DM in the sample.

Another unexpected result was the plateauing of long chain acylcarnitine concentrations between 12 and 24 weeks. It is unknown exactly when homeostasis of long chain acylcarnitine in the mitochondria occurs. However, our dat suggest that it may occur between 12 and 24 weeks of treatment.

Strengths

The strengths of this study include establishment of risk factors for altered carnitine metabolism; the fact that a randomized, double-blind, placebo controlled methodology was used; and statistically significant improvements in three domains of the SF36 and in synthetic erythropoietin doses in the treatment group. The establishment of risk factors was an original concept in the carnitine literature, currently no other reports of risk factors have occurred. However, there were reports of responders versus non-responders, indicating that a high level of variety from L-carnitine treatment was documented (11;18). A randomized, double-blind, placebo controlled study is the gold standard in

methodology for clinical trials. Although the sample was small, the methodology was stringent. Finally, significant results were found in the primary outcome variables indicating the hypothesis and methodology were sound and the aims appropriate. Another, strength, to this study was the level of volunteerism. The patients, nurses, pharmacist, and physicians that volunteered to assist with this study did so without any financial compensation. Participation without compensation in clinical trials is very rare.

Limitations

While this study had significant strengths, it also had limitations. During both phases I and II the sample size was too small for extensive multivariate analysis. This was unfortunate, especially since a major criticism of previous studies in the literature was their small sample size. A goal of the study was to obtain a large sample size and thereby overcome this weakness. This was done via power calculations which suggested a sample size of 50 in each group or higher. However, patient recruitment was more difficult than anticipated. Clinical trials are fraught with challenges and this study was no exception. Access to a sufficiently large population from which to screen and recruit the sample was unquestionably the most challenging. The Dialysis Center of Lincoln had over 200 patients. However, the strict inclusion criteria, especially >1 year on dialysis limited, the number of patients that could be included in the study. In CKD, patient mortality is increased compared to non-CKD patients, and co-morbidity is also significantly higher. Even after patients were included into the study, attrition due to death was high.

To ensure that patients were blind, L-carnitine for the treatment group was placed in an infusion bag with normal saline to mask drug odor and all members of the research team were blind to the randomization process except for Dr. Alan T. Davis who did not collect any data. However, no questionnaire was used to measure the effectiveness of these procedures.

Future Directions

The completion of this study is a platform for many other studies in both the clinical and metabolic areas. The risk factors that were established could be further clarified. Do all of the factors contribute the same weight in identifying alteration in carnitine metabolism or would do one or two make a larger contribution. Are there other factors that would aid in identifying altered carnitine metabolism that were not accounted for in this study? For example, would screening for an SF36 physical composite score of less than 35 capture a population with the same degree of alteration as was found in this study due to their severely diminished physical functioning?

Muscle biopsies were not feasible in this study.Hhowever, measurement of muscle carnitine content at different time points during L-carnitine treatment would result in better knowledge of muscle carnitine turnover, homeostasis, and tissue saturation. Measurements of muscle strength and fatigue could be measured in conjunction to determine associations with tissue carnitine concentration and muscle ability.

SF36 is a tool used to measure perceived quality of life and although it measures perceived physical ability, it is not an actual objective measure of physical functioning. Therefore, physical functioning measures such as muscle strength and fatigue, (sit-to-stand, six-minute walk, and gait speed) could be measured pre and post intervention in a randomized, double-blind, placebo controlled trial with intravenous L-carnitine treatment. These items have been shown to have a high correlation to mortality, hospitalization rates, independent living, and perceived quality of life (19-22). Therefore, improvement in these parameters would be a significant contribution to the CKD literature.

The increase in plasma acylcarnitine concentrations in the treatment group needs further investigation. It was hypothesized that the increase was due to mass action from the mitochondria. However, this has not clearly been established. Using isotopic tracers, carnitine could be tagged and measured after homeostasis in the plasma is reached. The tracers would reveal how much of the free carnitine was acylated and what lengths of fatty acyl moieties were attached.

In summary, in a defined sample of CKD patients receiving hemodialysis, after treatment with intravenous L-carnitine, there were improvements in total, free and acylcarnitine moieties, the SF36 domains: role-functioning, bodily pain, and role-emotional, and in synthetic erythropoietin dosages. Therefore, intravenous L-carnitine might be particularly benefical as an adjunct to treatment in patients receiving hemodialysis, especially if defined risk parameters are present.

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Appendixes

Appendix A

INFORMED CONSENT FORM

Phase I

For the participants of the

Carnitine Status in Patients Receiving Hemodialysis Study

Investigators: Alison Steiber MS, RD
(517) 355-9893

Lorraine Weatherspoon Ph.D, RD
(517) 432-0813

Alan Davis, Ph.D
(616) 454-9960

Project Description: This project is designed to assess carnitine levels in patients receiving hemodialysis and the role of carnitine in factors such as: age EPO dose, Mannitol dose, exercise, SF36 scores and protein intake. You may refuse to continue participation in this study at any time without any adverse effects on your medical care.
Confidentiality: Your identity will not be reported in any paper or document produced from this research. During and after this research, your privacy will be protected to the maximum of the law.
Procedures: Participation in this study will involve you allowing a small sample of the blood drawn, during your routine blood draw at the beginning of the month, to be used for analysis of carnitine. **It will not cost you anything and it will not require any additional "pokes" by needles.** With your permission the following information will be taken from your chart and used for analysis: age, EPO prescription, mannitol use, exercise data, most recent SF36 score, treatment information and health history. This information will be sent by mail to Michigan State University for analysis by the primary investigator.
Risks and Benefits: There are no risks or discomforts, beyond routine dialysis, associated with this research. There are no implied benefits from participating in this study.
Participant's rights as Human Subjects of Research:
Research subjects are encouraged to contact Dr. David E. Wright Chair of the University Committee on Research Involving Human Subjects, at Michigan State University (517) 355-2180, if you have any questions regarding your rights in this study.
Statement of Agreement: Please indicate your agreement with the contents of this consent form by signing and dating below.

_____ _____

Signature of Research Participant ***Date***

Consent Form

For Participants in the

Supplementing Hemodialysis Patients with L-Carnitine: Effect on Clinical Parameters and Quality of Life

Investigators: Lorraine Weatherspoon PhD, RD
(517) 432-0813

Alison Steiber MS, RD
(517) 432-0870

Alan Davis, PhD
(616) 454-9960

Leslie Spry MD
1(800) 927-2618

Jay Wish, MD
1(216) 844-3163

PROJECT DESCRIPTION:

We plan to study the effects of taking L-carnitine after dialysis treatments on quality of life, parathyroid hormone concentrations, hemoglobin and hematocrit concentrations, Epogen® dose, muscle cramps, and weight gain between dialysis treatments. Previous studies have suggested the use of carnitine decreases muscle cramping and promotes muscle functioning, lowers Epogen® doses and raises serum hemoglobin and hematocrit concentrations, lowers serum triglycerides and increases perceived quality of life scores. Patients who can participate in this study are those who are greater than 18 years of age, receive hemodialysis three times per week, and have not taken L-carnitine within the last three months.

WHAT YOU WILL BE ASKED TO DO IF YOU PARTICIPATE:

Your voluntary participation in this study will entail the following:

1. Allowing the researchers access to your medical records to collect data on Epogen® dose, weight changes, hypertonic solution use and monthly laboratory results such as serum blood urea nitrogen, parathyroid hormone, hematocrit, and hemoglobin.

2. Allowing a registered dietitian to perform 2 food histories on you at the beginning of the study and the end, the food histories will take approximately 15 minutes each,
3. Allowing a registered dietitian to perform a brief physical including lightly touching your temples, shoulders, clavicle region, knees, and calves at the beginning and end of the study, this brief physical will take approximately 10 minutes each time,
4. Allowing a social worker to administer a quality of life questionnaire at the beginning and end of the study, the quality of life questionnaire will take approximately 45 minutes to conduct and will include questions regarding your feelings on interactions with family members, friends and co-workers and your feelings on your abilities to perform various daily tasks,
5. Allowing a nurse to keep track of your muscle cramps and weight gain while you are on dialysis and during the study time,
6. Allowing a nurse to take a portion of the routine blood drawn each month to be analyzed for carnitine concentrations.
7. During the time of the study, you would receive either a placebo or L-carnitine after each dialysis treatment. Both the placebo and the L-carnitine will be given in your dialysis arterial-venous access, with no additional pokes with a needle required

You will be randomized to treatment or placebo. Randomization means that you will be placed into a group by chance, like flipping a coin. Neither you nor your study doctor will chose what group you will be in and you will have an equal chance of being placed in either group. Placebo refers to the group that will not receive carnitine, and instead will receive an inactive agent. There will be approximately 63 patients in each group.

The study will take 6 months to complete and it will not cost you or your insurance anything. The L-carnitine will be provided free of charge as will the laboratory tests for carnitine. L-carnitine will given in doses of 20mg/kg of dry body weight/day. The pills will be taken with liquid immediately following dialysis treatment on dialysis days and in the morning with breakfast on non-dialysis days.

YOUR PARTICIPATION IN THIS PROJECT IS VOLUNTARY:
You may refuse to continue participation in this study at any time. There is no penalty and your medical care will not be altered at all for withdrawing from this study.

YOUR PARTICIPATION IN THE PROJECT WILL BE KEPT PRIVATE:
During and after this research, your privacy will be protected to the maximum extent of the law. **Results of this study will not identify any individual.** Each subject will be assigned a subject number and in analyzing

the results from this study the data will be entered by subject number instead of the patients name to insure privacy. The bags which will be given to the patients participating in this study will be labeled with the patients name and subject number. All results will be kept in a locked file in the primary researchers office where only the primary researchers have the key. This study will be a double blind study where neither the primary researchers nor the nurses will know which patient is receiving the L-carnitine and which is receiving the placebo. Furthermore, the nurses administering the pills will not be the researchers collecting the data; therefore they will not have access to the individual results. The records and results of this study may be accessible to representatives of the Food and Drug Administration and the Combined Institutional Review Board.

Potential Benefits and Hazards:

RISK AND BENEFITS:

Some potential benefits from the carnitine supplementation may be increased quality of life, decreased parathyroid hormone concentrations, increased hemoglobin and/or hematocrit concentrations, decreased Epogen® dose, and decreased muscle cramping and fluid weight gain between dialysis treatments. If desired, you may learn of the study results by asking the one of the investigators for the results to available to you when they are completed.

Taking L-carnitine long term may cause transient nausea and vomiting, body odor and/or gastritis. Large doses of L-carnitine do not result in toxicity, however may result in diarrhea. The dialysis process easily removes the L-carnitine from the serum preventing toxicities. In a study with L-carnitine supplementation in hemodialysis patients 27 out of 62 of the patients reported some flu-like symptoms, 35 out of 62 reported headaches, 27 out of 62 reported sore throat, and 21 out of 62 reported high blood pressure, however it is not known whether these symptoms are related to the L-carnitine supplementation. Mild muscle weakness has been described only in uremic patients receiving D, L-carnitine which will not be used in this study. Seizures have occurred in persons with or without pre-existing seizure activity, and an increase in seizure frequency has been reported. Valproic acid is a medication given to children prone to seizures. Valproic acid has been reported to lead to carnitine deficiency. Therefore the children receiving valproic acid for their seizures may also be receiving carnitine therapy. Therefore no reports of seizures by hemodialysis patients receiving carnitine therapy have been documented.

RISK OF PHYSICAL INJURY:

If you are injured as a result of your participation in this research project, the Dialysis Center of Lincoln will provide emergency medical care if necessary. You will not be held responsible for any medical expenses incurred as a result of this injury. All such medical expenses will be paid by Michigan State University.

YOUR RIGHTS AS HUMAN RESEARCH PARTICIPANTS:

Participants in this study are welcome to call any of the study investigators with questions. Phone numbers for Dr. Weatherspoon, Mrs. Steiber and Drs. Davis and Spry are listed at the top of the Consent Form. You are encouraged to contact Dr. Ashir Kumar, Chair of the University Committee on Research Involving Human Subjects, at (517) 355-2180 or the Combined Institutional Review Board in Lincoln, NE (402) 486-7144 with any questions regarding your rights as a human subject raised by participation in this study. You are encouraged to ask one of the investigators about any specific questions regarding the research project.

STATEMENT OF AGREEMENT:

Please indicate your agreement with the contents of this consent form by signing and dating below.

_____

Signature_____ **Date**_____

Witness_____ **Date**_____

UCRIHS APPROVAL FOR
THIS project EXPIRES:

AUG 2 2003

SUBMIT RENEWAL APPLICATION
ONE MONTH PRIOR TO
ABOVE DATE TO CONTINUE

Appendix B

Data collection Forms I and II

Phase I

Data Collection Form I

Subject #:_____

Date:_____

Total:_____, Free:_____, Acyl-carnitine:_____

DOB:_____, Sex: (circle) male/female, Wt:_____ kg, Ht: _____in

Time receiving HD: _____ (circle) months/years,

Treatment Time: _____ (circle) minutes/hours

Live in Lincoln: yes / no,

if no: Travel > 1 hour to dialysis: yes / no,

Married: yes / no,

if no: Live alone: yes / no: Work: yes / no,

Latest SF36 Score:_____, History of ETOH Abuse: (circle) yes/no

<u>Exercise</u> for at least 1x/mo.: yes/no,

if yes: Ave RPEfor current week: _____, Ave PD for current week:_____,

<u>Blood Pressure</u>: Systolic:_____, Diastolic:_____

<u>Labs</u>: Alb:_____, BUN:_____, Chol:_____, Creat:_____, Ferritin:_____,

Hct:_____, Hgb:_____, PTH:_____, TG:_____, URR:_____, Al:_____,

Pre-alb.:_____, Iron:_____,

<u>Meds</u>: circle any that apply:

oral contraceptives, beta-blockers, salicylates, steroids, thiazidic diuretics,

benzodiazepines, clonidine, cimetidene, eparin, thiroyd homes, phenylidantoyne ;

EPO dose/treatment:_____, Length of time on EPO_____;

List any lipid lowering

drugs:_____

Patient is on an Al-based phosphate binder: yes/no

Mannitol: yes / no,

if yes: Ave. dose over the past month?_____ ,

<u>Co-morbidities</u>: circle any that apply:

Type 1 Diabetes, Type 2 Diabetes, Inflamatory Disease (Rheumetoid Arthritis, Crohns, Ulcerative Colitis, Asthma, COPD), Lupus, Current Cancer, History of Cancer, Current Infection, HTN,

other:_____

If yes for cancer,

type:_____

<u>Cardiac dysfunctions</u>:

CHF: yes/no,

LVH: yes/no; if yes: end diastolic diameter_____, left artrial dialation _____,

Ejection Fraction_____%

Other:_____

_____

Data Form II

Phase I

Dietary 24 Hour Recall

Dietitian:_____,

Patient:_____

AM:

snacks

Afternoon:

snacks

Pm:

snacks

Totals: Protein:_____, Kcals:_____, Carnitine:_____

Data Collection Forms

Phase II

Subject Number:_____ Date:_____

Anthropometric data: Date:

TSF:_____ _____ _____ Ave TSF:_____ (cm) _____
MAC:_____ (cm) _____
MAMC: MAC - (π x TSF) =_____ _____
Height_____ _____
Weight_____ BMI:_____ _____

Demographic Data:

Age:_____ _____
Primary etiology:_____ _____ _____

Co-morbidities: Check any that apply

1. Hypertension _____
2. Cardiovascular disease _____
3. LVH _____
4. Active Cancer _____
5. History of Cancer _____
6. Active Infection _____
7. Crohn's _____
8. Ulcerative Colitits _____
9. Diabetes: type 1 or 2 _____
10. Lupus _____
11. HIV/AIDS _____
12. Other:_____

EF %_____ LAD:_____

Dialyzer type:_____ Duration of txt:_____
Dialysis start date:_____

Laboratory Values: Date:

nPCR _____ _____
BUN _____ _____
PTH _____ _____
Alb _____ _____
TG _____ _____
HDL (most recent draw) _____ _____
Hct _____ _____
Hgb _____ _____

Medications:

Aspirin yes:_____ no:_____ _____
Lipid lowering Medications yes:_____ no:_____ _____
(lipitor, tricor, zocor, questran,...) yes:_____ no:_____ _____
Epogen yes:_____ no:_____ _____
dose _____ _____

Daily Nursing flow sheet

Phase II Carnitine Trial

Day/Date:_____

Patient Number: _____

Patient Name: _____

Patient exercising? YES_____ NO_____

Pre-dialysis weight: _____, Post-dialysis weight: _____

Patient receiving hypertonic solution? YES_____ NO_____

If YES: Amount_____

Is patient having MUSCLE CRAMPING? YES____, NO_____

If YES: NUMBER of cramps during dialysis:_____

Were the cramps (circle one):

MILD MODERATE STRONG VERY STRONG

Is patient on Epogen®? YES____ NO____

If yes: Amount per kg: _____

SF-36 HEALTH SURVEY

INSTRUCTIONS: This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is:

(circle one)

Excellent 1

Very good 2

Good 3

Fair 4

Poor 5

2. Compared to one year ago, how would you rate your health in general now?

(circle one)

Much better now than one year ago 1

Somewhat better now than one year ago 2

About the same as one year ago 3

Somewhat worse now than one year ago 4

Much worse now than one year ago 5

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(circle one number on each line)

ACTIVITIES	Yes, Limited A Lot	Yes, Limited A Little	No, Not Limited At All
a. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
b. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
c. Lifting or carrying groceries	1	2	3
d. Climbing **several** flights of stairs	1	2	3
e. Climbing **one** flight of stairs	1	2	3
f. Bending, kneeling, or stooping	1	2	3
g. Walking **more than a mile**	1	2	3
h. Walking **several blocks**	1	2	3
i. Walking **one block**	1	2	3
j. Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

(circle one number on each line)

	YES	NO
a. Cut down on the **amount of time** you spent on work or other activities	1	2
b. **Accomplished less** than you would like	1	2
c. Were limited in the **kind** of work or other activities	1	2
d. Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

(circle one number on each line)

	YES	NO
a. Cut down the **amount of time** you spent on work or other activities	1	2
b. **Accomplished less** than you would like	1	2
c. Didn't do work or other activities as **carefully** as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

(circle one)

Not at all 1

Slightly 2

Moderately 3

Quite a bit 4

Extremely 5

7. How much bodily pain have you had during the past 4 weeks?

(circle one)

None 1

Very mild 2

Mild 3

Moderate 4

Severe 5

Very severe 6

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

(circle one)

Not at all 1

A little bit 2

Moderately 3

Quite a bit 4

Extremely 5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks -

(circle one number on each line)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
a. Did you feel full of pep?	1	2	3	4	5	6
b. Have you been a very nervous person?	1	2	3	4	5	6
c. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
d. Have you felt calm and peaceful?	1	2	3	4	5	6
e. Did you have a lot of energy?	1	2	3	4	5	6
f. Have you felt downhearted and blue?	1	2	3	4	5	6
g. Did you feel worn out?	1	2	3	4	5	6
h. Have you been a happy person?	1	2	3	4	5	6
i. Did you feel tired?	1	2	3	4	5	6

10. During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting with friends, relatives, etc.)?

(circle one)

All of the time 1

Most of the time 2

Some of the time 3

A little of the time 4

None of the time 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

(circle one number on each line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
a. I seem to get sick a little easier than other people	1	2	3	4	5
b. I am as healthy as anybody I know	1	2	3	4	5
c. I expect my health to get worse	1	2	3	4	5
d. My health is excellent	1	2	3	4	5

24 hour recall "Multiple Pass Method"

Date:_____, Subject number:_____

Time/Meal	Food Consumed	Amount