

Biochemical Parameters, Acylcarnitines, Correlate with Nutrition and Clinical Outcomes in Hemodialysis Patients

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ABSTRACT:

Background: Patients on hemodialysis (HD) may experience decreased nutritional and quality of life status. Previously when carnitine, a molecule involved in lipid metabolism, was supplemented in hemodialysis patients it was shown to improve nutrition parameters, albumin and body mass index. Carnitine bound to fatty acid chains of different lengths can reflect mitochondrial metabolism. For instance, increased concentrations of long chain acylcarnitines (AC) may reflect a defect in beta oxidation.

Hypothesis & Purpose: We hypothesized that increased concentrations of medium and long chain AC would have a negative impact on nutrition and physical function parameters in hemodialysis patients. The purpose of this study was to examine the associations between commonly measured nutrition and physical function parameters and AC.

Methods: This was an analysis of baseline data from a randomized, double blind, placebo-controlled study conducted in hemodialysis patients in the Midwestern USA. Patients included in this study met the following criteria: ≥ 19 years of age, dialysis ≥ 6 months, are currently receiving low acetate hemodialysis treatments 3 times a week for a minimum of 3 hours, a Medical Outcomes Short Form (SF36) Physical Composite Score (PCS) of < 40 , and a plasma free carnitine concentration of $< 40 \mu\text{mol/L}$. A rolling recruitment strategy was used to enroll patients into the study. Data collected were: plasma AC, nutrition status (7 pt Subjective Global Assessment (SGA)), serum albumin and a sub-group of serum pre-albumin parameters, and functional status parameters (sit-to-stand (STS), gait speed (GS), and a sub-group of 6 minute walk (6MW)). AC was measured from plasma at baseline using MS/MS; parameters were measured within 2 weeks of the plasma collection. SPSS vs. 17.0 was used to analyze the data and significance was defined as $p < 0.05$. Patients with missing data were included in some of the analyses thus the sample size ranges from a total of 65 to 60 depending on the variable analyzed. Sub-group analysis was conducted on 40 subjects.

Results: Of the patients studied ($n=65$) 42% were female, 74% were Caucasian, 4% were Hispanic and 21% were African American; 48% had a primary etiology of diabetes, 34 had hypertension and 18% had other primary etiologies. The mean values were age 68.5 ± 14.0 years, albumin $3.8 \pm 0.4 \text{ mg/dL}$, pre-albumin $24.7 \pm 7.2 \text{ mg/dL}$, STS 5.3 ± 4.2 reps., GS 7.8 ± 4.5 seconds, 151.3 ± 17.7 ft. From SGA, no patients were severely malnourished, 56% of the patients were moderately malnourished and 44% were mildly malnourished or well nourished. Spearman's correlation was used due to the skewed nature of the AC to assess the relationship between AC and the nutritional and physical function parameters. The AC that were significant with nutrition parameters were: serum albumin and the short chain (SC) AC propionylcarnitine ($r=0.29$), and butyrylcarnitine ($r=0.32$), 2-methyl-butylcarnitine ($r=0.32$), and the long chain (LC) AC linoleoylcarnitine ($r=0.27$); prealbumin and the LCAC trans-2-hexadecenoylcarnitine ($r=0.36$) and the dicarboxylic acid (DA) suberoylcarnitine ($r=0.45$); SGA and the medium chain (MC) AC cis-4-decenoylcarnitine ($r=0.40$) and the LCAC trans-2-tetradecenoylcarnitine ($r=0.34$). The AC that were significant with physical function parameters were: STS and the SCAC butyrylcarnitine ($r=-0.27$), the MCAC lauroylcarnitine ($r=-0.30$), trans-2-dodecenoylcarnitine ($r=-0.25$), myristoylcarnitine ($r=-0.28$), the LCAC palmitoleoylcarnitine ($r=-0.30$), and oleoylcarnitine ($r=-0.29$); GS and the MCAC cis-4-decenoylcarnitine ($r=0.32$), and the DA 3-methyl-glutaroylcarnitine ($r=-0.28$); 6MW and the MCAC cis-4-decenoylcarnitine ($r=-0.50$) and trans-2-dodecenoylcarnitine ($r=-0.33$), and the LCAC palmitoleoylcarnitine ($r=-0.31$), palmitoleoylcarnitine ($r=0.50$), oleoylcarnitine ($r=-0.38$), linoleoylcarnitine ($r=-0.43$), ethyl-malonoylcarnitine ($r=-0.32$), and the DA adipoylcarnitine ($r=0.39$) and 3-methyl-glutaroylcarnitine ($r=0.42$).

Conclusion: In conclusion the nutrition parameters are predominately positively associated with AC; while physical functioning and AC are predominately negatively correlated. These data support the original hypothesis for physical functioning where higher AC correlate with worse physical function; however the hypothesis for nutritional parameters is not supported by these data.

Background:

- In chronic kidney disease (CKD) there is an altered carnitine homeostasis (Ricanati, Tserng, Hoppel, 1987)
 - ◆ in the urine of healthy subjects
 - 98-99% free carnitine reabsorbed & $5 \mu\text{mol/kg/day}$ of acyl + free lost in urine/day (Lombard et al, 1989)
 - ◆ in the dialysate of patients on dialysis due to chronic kidney disease (Evans et al, 2000)
 - Approximately $1050 \mu\text{mol/week}$ lost (equal amounts of free & acyl)
- Patients receiving dialysis due to chronic kidney disease (CKD) have persistent altered plasma carnitine status
 - ◆ Acylcarnitine to free carnitine ratios > 0.8
 - ◆ Free plasma concentrations below normal
 - ◆ Acylcarnitine (although not acetyl) above normal
- A sub-group of dialysis patients may be at an ↑ risk for altered carnitine concentrations (Steiber et al, 2004)
- In patients with CKD
 - ◆ β -oxidation may be altered leading to an accumulation of medium chain- & long chain AC moieties in the mitochondria that are not found in healthy subjects (Hoppel et al, 2002)
 - ◆ Long chain AC are known to have deleterious effects on cellular structure & function

Hypothesis:

- We hypothesize that increased concentrations of medium & long chain AC have a negative impact on nutrition and physical function parameters in HD patients. Therefore, the purpose of this study is to examine the association between commonly measured nutrition and physical function parameters and AC.

Materials & Methods:

- Cohort
 - ◆ The patients for the study were treated at the Dialysis Center of Lincoln, NE and its constituent satellite sites as well as DaVita Clinics in Cleveland, Ohio, USA.
 - ◆ The criterion for patient selection include:
 - Inclusion criteria:
 - ✓ Age ≥ 19 years
 - ✓ At least three hours of low acetate hemodialysis treatments three times per week
 - ✓ Received dialysis ≥ 6 months
 - ✓ MOS SF36 PCS < 40
 - ✓ Plasma free carnitine $< 40 \text{ mol/L}$
 - Exclusion:
 - ✓ Previous treatment with L-carnitine within 6 months of study
 - ✓ Excessive blood loss within two months of study
 - ✓ skeletal muscle function diseases
 - ✓ liver disease
 - ✓ pregnancy

Figure 1. Flow of subjects from screening to end of trial, data for this analysis from baseline only.

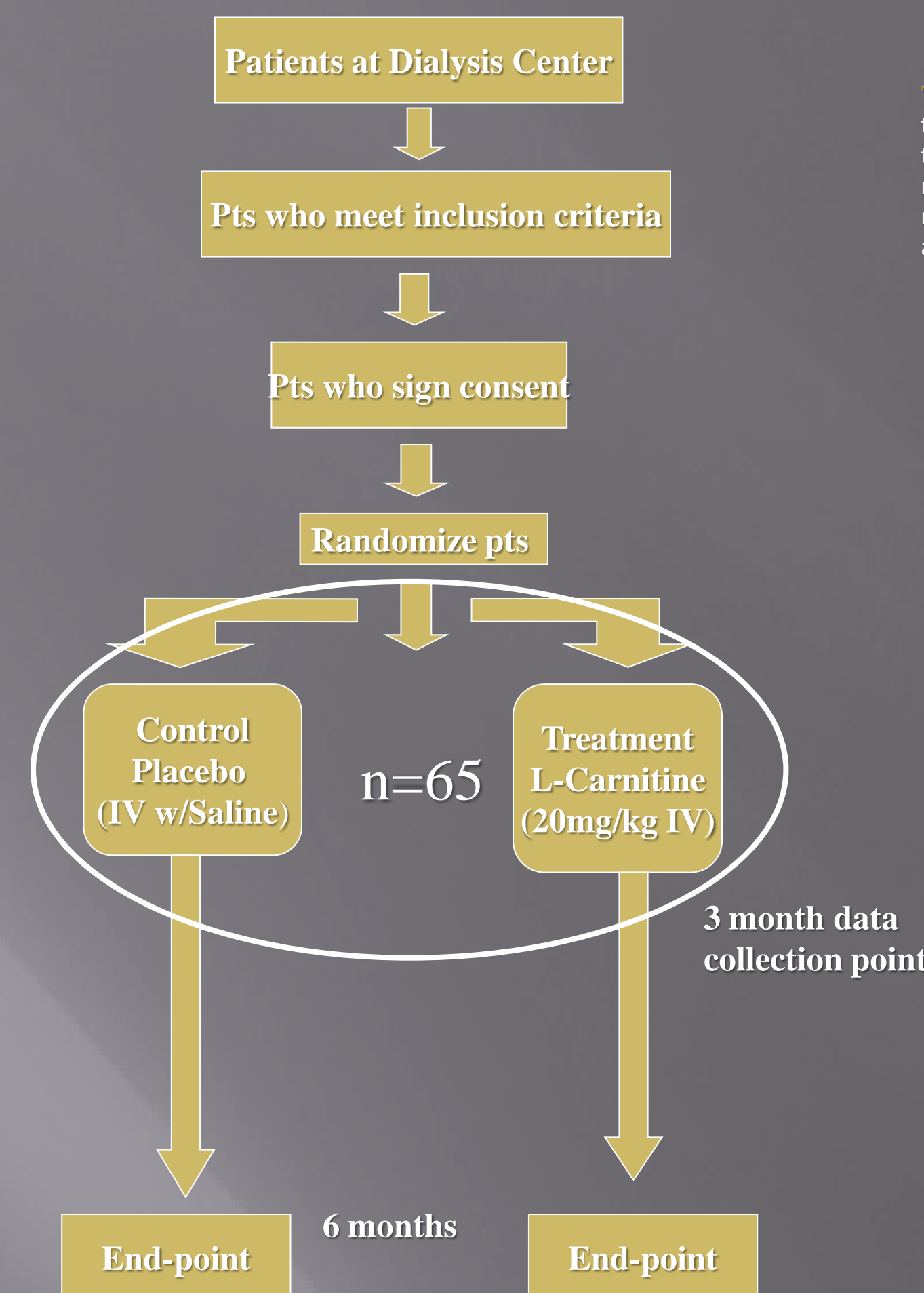


Table 1. AC moieties and the significant correlations to physical function measures. * These moieties are dicarboxylic acid AC

Acylcarnitine Moieties and Functional Status:

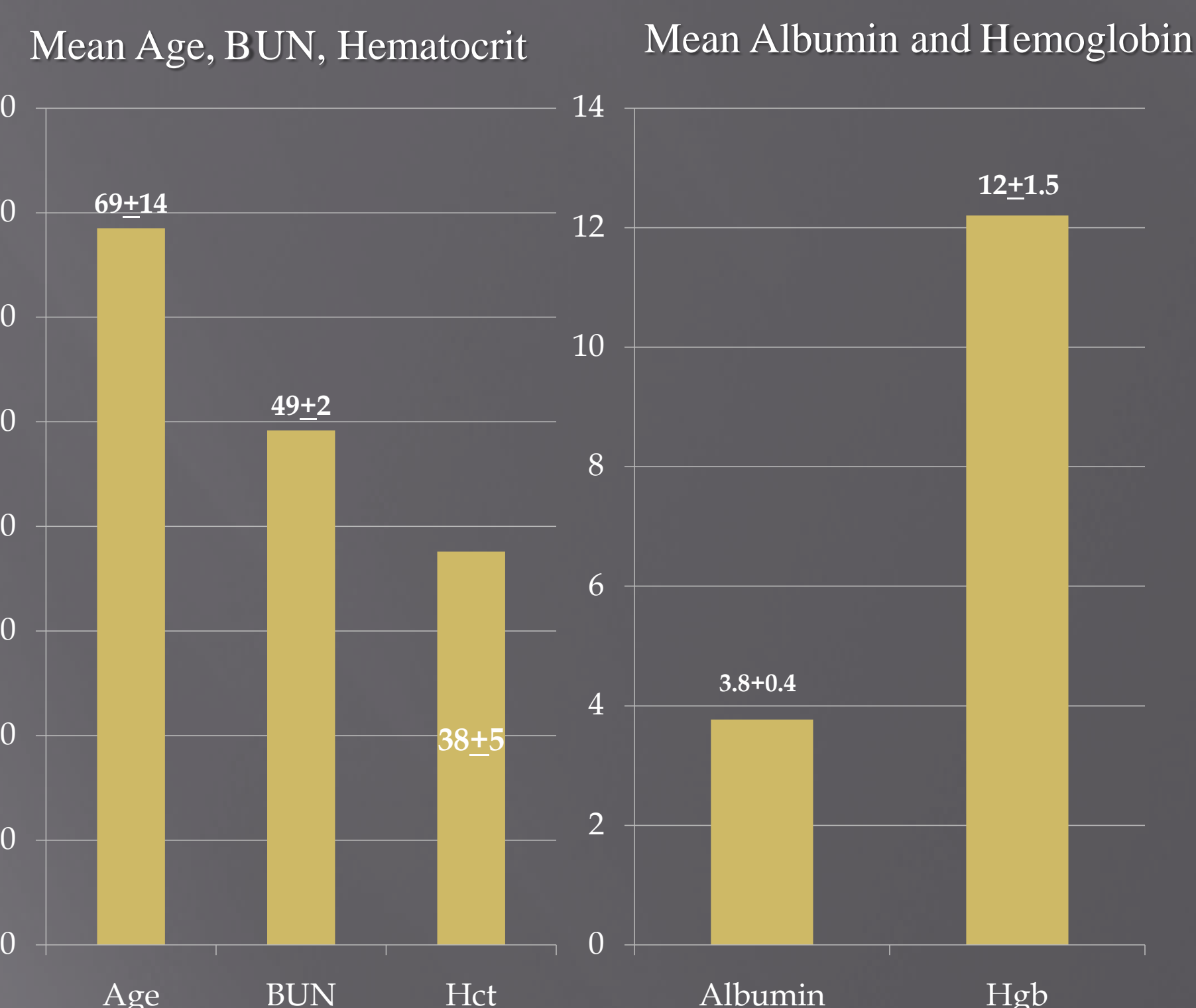
Acylcarnitines	Sit-to-stand	Gait Speed (faster is better)	6 min walk
butyryl	-0.27		
lauroyl	-0.3		
cis-4-decenoyl		0.32	-0.50
trans-2-dodecenoyl	-0.25		-0.33
myristoyl	-0.28		
palmitoleoyl	-0.30		-0.50
oleoyl	-0.29		-0.38
linoleoyl			-0.43
ethylmalonyl			-0.32
*adipoyl			0.39
*3-methyl-glutaroyl		-0.28	0.42

Acylcarnitines	Albumin	Pre-albumin	Subjective Global Assessment
Propionyl	0.29		
butyric	0.32		
2-methyl-butyl	0.32		
cis-4-decenoyl			0.40
trans-2-tetradecenoyl			0.34
trans-2-hexadecenoyl		-0.36	
linoleoyl	0.27		
*suberoyl		0.45	

Table 2. AC moieties and the significant correlations to nutrition parameters. * These moieties are dicarboxylic acid AC

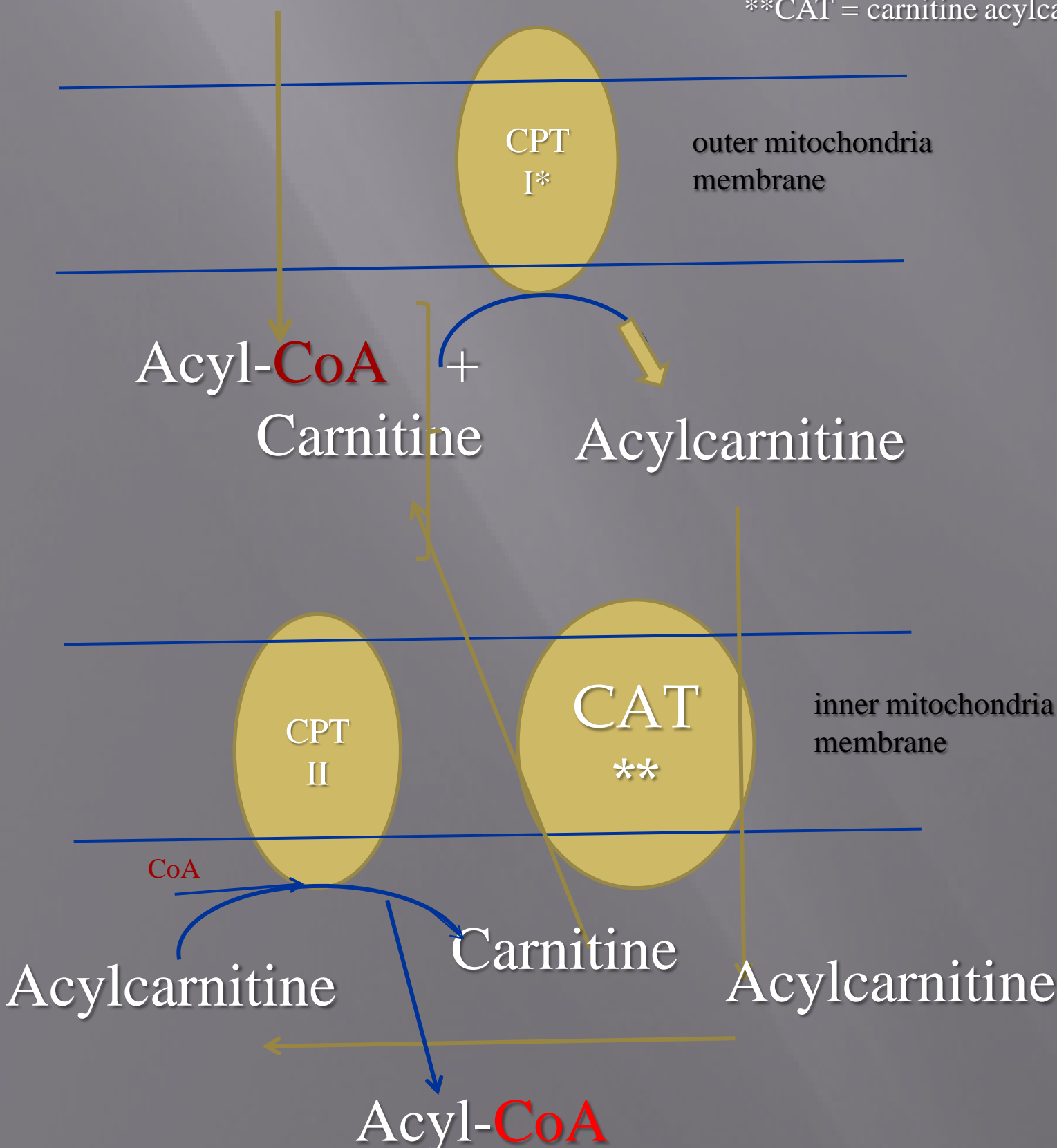
- Data:
 - ◆ Collected from pt interview, medical records, nursing flow sheets, laboratory reports
 - ◆ 1 mL aliquot plasma collected via patient access prior to dialysis
 - Sample frozen at -80° until analysis
 - ◆ Total and free carnitine & acylcarnitine
 - Analyzed by HPLC/ESI/MS/MS following derivatization with pentafluorophenacyl trifluoromethanesulfonate (Minkler et al, 2005)
 - ◆ Statistical
 - Test for normalacy
 - Descriptive analysis & Spearman's correlations conducted on SPSS vs 17.0

Select Baseline Characteristics (n=65)



Acyl-CoA (fatty acid chain)

*CPT = carnitine palmitoyl transferase
**CAT = carnitine acylcarnitine translocase



CONCLUSIONS:

- The nutrition parameters are predominately positively associated with AC; while physical functioning and AC are predominately negatively correlated. These data support the original hypothesis for physical functioning and pre-albumin where higher AC correlate with worse physical function; however the hypothesis for albumin and SGA is not supported by these data.
 - ◆ Physical Functioning:
 - Short chain AC indicate that β -oxidation occurred to some extent.
 - Positive correlations between these moieties and physical functioning indicate that these metabolized acyl chains are supporting activity.
 - Negative correlations between medium and long chain AC indicates that these moieties have incomplete β -oxidation.
 - Positive benefits seen in the dicarboxylic acids indicate that acyl chain oxidation in the peroxisomes may benefit the physical functioning.
 - ◆ Nutrition Parameters:
 - Data for Albumin & SGA did not support hypothesis in the manner similar to physical functioning.
 - Pre-albumin data did support hypothesis similar to the physical function data.
 - More research is needed for further understanding.

Acknowledgements:

We thank DaVita Clinical Research® for providing part of the clinical data obtained for this research project and Sigma Tau Pharmaceuticals, Inc for providing a grant to conduct this study.