Altered carnitine metabolism in dialysis patients with reduced physical function may be due to dysfunctional fatty acid oxidation

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Abstract

Background. It has been reported that hemodialysis patients have elevated plasma acylcarnitine concentrations, which correlates to reduced red blood cell integrity. It has also been reported that the supplementation of L-carnitine for these patients improves anemia, glucose metabolism and muscle function, but the mechanism of these relationships remains unknown. We hypothesized that the cause of increased plasma acylcarnitines is incomplete fatty acid oxidation and the underlying disturbance of metabolism reduces muscle function, resulting in decreased ability to function and quality of life, and glucose availability, resulting in decreased red blood cell integrity and worsened anemia.

Methods. This analysis was conducted on baseline data from a clinical trial of carnitine supplementation in hemodialysis patients with reduced physical function and free carnitine levels. Partial correlations controlling for age, gender, hemoglobin and subjective global assessment score for each acylcarnitine species and outcome were computed using SPSS version 17.0 and a significance level of P < 0.05. To measure the impact of acylcarnitine acyl chain length on these relationships, the correlation coefficients were categorized by chain length and linear regressions were computed for each outcome measure.

Results. Linear regression analysis (n = 58) revealed significant negative relationships between chain length and Short Form-36 physical composite score, sit-to-stand count and 6-min walk distance ($r^2 = 0.635, 0.332$ and 0.347, respectively) and a significant positive relationship with erythropoietin dose ($r^2 = 0.181$).

Conclusion. Our data revealed that longer acyl chain length significantly predicts poorer physical function and worsened anemia, and this data supports our proposed mechanism, which may lead to increased understanding of altered carnitine metabolism in hemodialysis patients.

Keywords: acylcarnitine; fatty acid oxidation; hemodialysis; inflammation; physical function

Introduction

Chronic kidney disease (CKD) is a condition associated with high rates of mortality [1, 2]. The strongest outcome predictors in CKD patients are malnutrition, inflammation [3] and anemia [4]. Optimal care of patients with CKD should involve identifying and targeting the causes of all these symptoms.

One well-documented phenomenon in hemodialysis patients that may relate to these outcome predictors is elevated levels of a variety of plasma acylcarnitines [5, 6]. Acylcarnitines are the product of enzymatic esterification of carnitine, a water-soluble molecule vital for the transport and oxidation of fatty acids [7]. In healthy individuals, the primary function of these compounds is to transport fatty acids between the peroxisomes (where very long-chain fatty acid oxidation begins) and the mitochondria (where fatty acid oxidation is completed) [7]. However, acylcarnitines are found in the plasma under pathologic conditions such as genetic fatty acid oxidation disorders [8], in which the presence of an individual acylcarnitine species corresponds to a specific enzyme deficiency [9] and Type 2 diabetes mellitus [10]. It is thought that the appearance of acylcarnitines in the plasma is a result of the antiprotein enyme carnitine-acylcarnitine translocase shuttling excess partially oxidized fatty acids out of the mitochondria in order to maintain free Coenzyme A levels in the mitochondrial matrix [7]. These acylcarnitines have been demonstrated, in vitro, to activate inflammatory pathways [10], and previous studies in hemodialysis patients have reported that elevated levels of plasma acylcarnitines correspond to decreased red blood cell integrity [11] and increased erythropoiesis-stimulating agent (ESA) resistance [12].

Because of the evidence of altered carnitine metabolism provided by the presence of plasma acylcarnitines, the therapeutic supplementation of carnitine in hemodialysis patients has been studied and has shown improvements in anemia, metabolism and skeletal muscle function. A 2002 meta-analysis of carnitine therapy in dialysis patients [13] found that, in three studies predating ESA therapy, carnitine...
treatment resulted in a significant increase in hemoglobin concentration and, in six more modern studies, treatment resulted in significantly reduced ESA doses, which indicates improved anemia. Carnitine supplementation has also been shown to increase glucose disposal in healthy subjects [14] and Type 2 diabetic patients [15], but an investigation into the effects of carnitine therapy on metabolism in dialysis patients [16] demonstrated this increased glucose disposal only in the subjects with the highest insulin resistance. However, a significant protein sparing effect was observed in the treatment group. Lastly, a study of skeletal muscle morphology and function in dialysis patients [17] found that all subjects suffered from muscle atrophy at baseline and that treatment with carnitine reversed atrophy in aerobic muscle fibers and improved muscle function.

Due to the growing evidence of the benefits of carnitine supplementation in dialysis patients, its use has been recommended for the treatment of anemia that is resistant to erythropoietin therapy, chronic intradialytic hypotensive episodes, cardiomyopathy and skeletal muscle weakness [18]. However, the National Kidney Foundation Kidney Disease/Dialysis Outcomes and Quality Initiative (K/DOQI) does not currently recommend carnitine supplementation for all dialysis patients due to a lack of high quality trials and because ‘no pathogenic mechanism by which carnitine deficiency might contribute to anemia . . . has been conclusively elucidated’ [19].

This study aims to develop a hypothesis for the mechanisms of altered carnitine metabolism and its treatment by carnitine supplementation which would include the existing evidence and hold up to analysis of baseline data from an ongoing clinical trial. Based on data from studies of inborn errors of metabolism, the presence of plasma acylcarnitines suggests a dysfunction in the metabolism of fatty acids [20]. In a state of impaired fatty acid oxidation, muscle fibers that normally depend on fat for energy would have reduced ability to function and would consume more glucose. Increased consumption in the muscles could limit the availability of glucose for red blood cells, which are entirely dependent on glucose for energy, and undernourished red blood cells would be more likely to lyse, contributing to anemia and ESA resistance. Under these circumstances, glucose utilization increases, which explains why dialysis patients did not show the same improvement in glucose utilization as non-dialysis patients when treated with carnitine. Additionally, the acylcarnitines could be irritants responsible for an inflammatory response as demonstrated by Adams et al. [10], and this inflammation contributes to the observed anemia and impaired muscle function.

Carnitine supplementation would alleviate the symptoms by providing free carnitine for the carnitine-acylcarnitine translocase enzyme, thus transporting incompletely oxidized fats out of cells as acylcarnitines at an increased rate. The removal of acylcarnitines from the cells would terminate any inflammatory signal that the acylcarnitines generate and allow for partial oxidation of fatty acids to continue. Resumed fatty acid oxidation would improve the function of the muscles by providing more energy and decrease the muscles’ dependence on glucose. Decreased muscular glucose use would increase glucose availability for red blood cells, which would improve their viability, and reduce the necessity of proteolysis for gluconeogenesis.

These proposed mechanisms include the documented relationships between acylcarnitines and anemia [12] and inflammation [10]. They also include the improvements in anemia [11] and muscle function [17], the lack of improvement in glucose uptake [16] and the decrease in proteolysis [16] that have been documented with carnitine supplementation.

Therefore, we hypothesize that the cause of increased plasma acylcarnitines is incomplete fatty acid oxidation, that the acylcarnitines cause inflammation and that the underlying disturbance of metabolism reduces muscle function, resulting in decreased ability to function and related quality of life and glucose availability, resulting in decreased red blood cell integrity and worsened anemia. This hypothesis was tested in a sample of hemodialysis patients with elevated plasma acylcarnitines and reduced physical function by examining the statistical relationships between measured plasma acylcarnitine moieties and outcome measures related to inflammation, physical function and quality of life and anemia, and the expected result was that increased acylcarnitine concentrations would correspond to poorer outcomes.

Materials and methods

This cross-sectional analysis, using data collected at baseline from an ongoing clinical trial, adheres to the Declaration of Helsinki and was approved by the institutional review board of University Hospitals Case Medical Center as an addendum to the parent study. In the parent study, informed consent was obtained before any study-related procedures began. The parent study was a randomized placebo-control clinical trial on the efficacy of L-carnitine supplementation in hemodialysis patients. The primary outcomes of the parent study were Medical Outcomes Trust Short Form (SF)-36 domain and composite scores, physical function measures and synthetic erythropoietin dose. Subjects for the parent study were verbally recruited from the Dialysis Center of Lincoln in Lincoln, Nebraska and the DaVita Dialysis Centers of the greater Cleveland, Ohio area. Subjects were eligible for the study if they were at least 19 years old, had been receiving hemodialysis for at least 6 months, received at least 3 h of low acetate hemodialysis at least three times/week, had an SF-36 physical composite score (PCS) <40 and had a plasma-free carnitine level <40 μmol/L. Subjects were excluded from the study if they had received L-carnitine treatment within 6 months prior to the study, severe blood loss within 2 months prior to the study, a primary disease that affects skeletal muscle function, severe liver disease or were pregnant. From the available data, 58 subjects had sufficient data to be included in this study.

Anthropometric data

Subjects’ age, gender, height, post-dialysis weight and comorbidities were collected from their medical records. The subjects’ body mass index (BMI) was calculated as the quotient of each individual’s post-dialysis weight (in kilograms) divided by the square of their height (in meters).

Biochemical data

Plasma samples were analyzed via ion-spray tandem Mass Spectrometry to determine the moieties of acylcarnitines present. Acylcarnitines derived from dicarboxylic acids or containing phenyl groups were not included in the analyses in this study. A portion of the collected plasma was transported to the University Hospitals Case Medical Center for analysis of C-reactive protein concentrations using the Dade Behring, Inc. CardioPhase® high-sensitivity C-reactive protein immunoassay in order to describe the occurrence and severity of clinical inflammation.
Physical function

The assessment of physical function of the subjects was performed prior to a dialysis treatment using a 6-min walk test for aerobic endurance and a sit-to-stand test for lower body muscle strength. These tests were performed at the subject’s dialysis center prior to receiving a dialysis treatment. These tests were conducted as described by Painter et al. [21]. Results from the 6-min walk tests are expressed in feet travelled, and results from the sit-to-stand tests are expressed in maneuvers performed per 30 s.

Medical Outcomes Trust SF-36

This questionnaire was performed on subjects during a dialysis treatment prior to the intervention. Scoring was done according to the Medical Outcomes Trust [22]. The SF-36 generates eight scores corresponding to different domains of self-perceived health-related quality of life, and each ranges from 0 to 100 in value. This analysis used the PCS, which was calculated according to the survey instructions from the four physical-related domains: vitality, physical functioning, bodily pain and physical-role functioning.

Other data collected

The subjects’ most recent recorded hemoglobin values and ESA (human recombinant erythropoietin) dose were collected from their respective medical records to describe the occurrence and severity of anemia. The ESA dose is reported in units administered per dialysis session. The nutritional status of the subjects was described using the 7-point Subjective Global Assessment form [23] and was completed by trained research assistants while the subjects received dialysis.

Data analysis

Data were analyzed using SPSS version 17.0 and statistical significance was defined as P < 0.05. We assessed the distribution of the primary outcome variables for this analysis. Descriptive statistics are presented as mean ± SD or as number and percentage where appropriate. Partial correlation coefficients were obtained for each acylcarnitine species and outcome measure (physical function test results, SF-36 PCS and C-reactive protein) while controlling for age, gender, hemoglobin and subjective global assessment score. Partial correlation was also performed for erythropoietin dose and acylcarnitine species, but hemoglobin was not included in this model due to its close relationship with ESA dose. The potentially confounding factors controlled for in the partial correlations are factors known to account for differences in the selected outcomes but were not necessarily significant in preliminary analyses performed with this data set. Partial correlations were calculated separately for each outcome measure, and subjects’ missing data for an outcome measure were excluded from only that particular calculation.

The resulting correlation coefficients were then categorized by the number of carbon atoms contained in each acylcarnitine’s acyl chain, and linear regression models were generated to determine the impact of acyl chain length on the relationship between acylcarnitines and each outcome measure. These regression models were used to create trend line equations and calculate their x-intercepts. Microsoft Excel 2010 was used to create the scatter plots with approximated trend lines used in Figure 1.

Results

The 58 subjects analyzed in this study were 68 ± 14.0 years of age and were 57% male. Complete descriptive statistics for demographic, clinical and outcome measures are presented in Table 1.

Including isomers, 27 unique acylcarnitine species were included in the analysis. These species included acyl chain lengths ranging from 2 to 18 carbons and included saturated, unsaturated and branched chains. No measureable quantities of very-long-chain acylcarnitines (chain length > 18) were found in our analyses. Partial correlations revealed significant relationships between eight of the acylcarnitine species and the sit-to-stand or SF-36 PCS outcome measures. Correlation coefficients for acylcarnitine species that were significantly related to at least one outcome measure are presented in Table 2. The remaining 19 acylcarnitine species presented no significant relationships.

Linear regressions between the length of acylcarnitine acyl chain and strength of relationship to each outcome measure (represented by the above-described partial correlation coefficients) revealed significant relationships for all five outcome measures. For each regression, the direction of the association relates longer acylcarnitine acyl chains with poorer outcomes. Table 3 presents the results of the linear regressions, and Figure 1 presents the corresponding scatter plots with trend lines.

In addition to the significant relationship between longer acylcarnitine acyl chains and poorer outcomes, the trend line equations present x-intercepts within the data range, suggesting that acylcarnitines with chain lengths less than the x-intercept are associated with improved outcomes. Table 4 contains the trend line equations and their calculated x-intercepts.

Discussion

In a study of baseline data from an ongoing clinical trial, we found that longer chain acylcarnitines correlate to poorer outcome measures related to inflammation, physical function and anemia. Conversely, we also found that shorter chain acylcarnitines correlate to more positive outcomes. One branch of our hypothesis—that plasma acylcarnitines cause inflammation—was not supported by our analysis. The other branches of our hypothesis—that plasma acylcarnitines are evidence of an underlying dysfunction in fatty acid metabolism which leads to decreased physical function and anemia—were supported by our data, but the relationships appeared to be more complex than anticipated. This increased complexity, however, may help lead to the identification of the metabolic dysfunction.

As can be seen by the inclusion criteria for this study, the patients who were recruited for had lower perceived quality of life scores and were older than what has been reported by the Dialysis Outcomes Practice Patterns Study [24]. Additionally, the patients had a mean BMI of 28 ± 6.6 kg/m² indicating overweight status with a mean subjective global assessment score of 5.2 ± 1.0 indicating moderate nutritional loss. This pattern reflects a patient sample with mildly excessive stores of adipose but an overall depleted nutritional status. The depleted nutritional status may be contributing to poor functional performance. Cheema et al. [25] reported muscle both quantity and quality correlated with nutrition indices in a recent study using computer tomography scans to assess muscle.
As seen in Table 2, some of the acylcarnitine species correlated significantly with the physical function outcomes measures; however, no single species correlated significantly across more than two outcome measures. If these compounds are indeed evidence of dysfunctional fatty acid oxidation, then the different acylcarnitine species cannot all be considered equally. Since fatty acid oxidation begins with longer acyl chains and sequentially shortens them to release energy, longer chain acylcarnitines would correspond to earlier failures in fatty acid oxidation and more severe symptoms, as is seen in inborn errors of metabolism [8].

In order to investigate the relationship between acylcarnitine chain length and our outcome measures, the partial correlation coefficients for individual acylcarnitine species were categorized by the number of carbon atoms contained in each acyl chain, and linear regression models were generated to determine the impact of acyl chain length on the relationship between acylcarnitines and each outcome measure. The results of these regressions indicated that acyl chain length significantly affects the impact that acylcarnitines have on our outcome measures, and that longer chains are negatively associated with physical function and related quality of life and positively associated with inflammation and anemia.

These results were consistent with previous reports that plasma acylcarnitines are associated with increased ESA resistance [12], but our results provided additional information by establishing the impact of acyl chain length on that relationship. To our knowledge, this study was the first to establish a relationship between plasma acylcarnitines and decreased physical function although previous studies have demonstrated that carnitine therapy improves physical function [6]. Unlike Adams et al. [10], we did not find significant relationships between medium-chain acylcarnitines and inflammation, and our data indicated that longer chain lengths were more strongly associated with inflammation. This difference in observations may have been due in part to the fact that Adams et al. used nuclear factor-kappaB as an indicator of inflammation while we used C-reactive protein, but both these measures of inflammation tend to increase together [26, 27]. The relationship that we observed between longer chain acylcarnitines and inflammation was likely not the result of the action of those acylcarnitines but rather another symptom of the underlying problem with fatty acid metabolism.

![Fig. 1. Scatter plots with trend lines between acylcarnitine chain length and strength of relationship (as represented by partial correlation coefficient) to outcome measures: (a) C-reactive protein, (b) 6-min walk distance, (c) sit-to-stand maneuver count, (d) Medical Outcomes Trust SF-36 PCS and (e) ESA dose.](image-url)
In addition to revealing the impact of chain length on the relationships between acylcarnitines and outcome measures, the regression equations all presented realistic x-intercepts. This suggests that the relationships between chain length and outcome measures reverse when the chain length is less than the x-intercept. More simply, longer chains are associated with negative outcomes, and shorter chains are associated with positive outcomes. This again was consistent with our hypothesis as shorter chain acylcarnitines would indicate more complete fatty acid oxidation and better outcomes.

**Table 1.** Descriptive statistics for demographic, clinical and outcome measures (*n* = 58)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>68.3 ± 14.0</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.0 ± 6.6</td>
<td></td>
</tr>
<tr>
<td>Gender Male</td>
<td>33 (56.9)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>25 (43.1)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity Caucasian</td>
<td>42 (72.4)</td>
<td></td>
</tr>
<tr>
<td>African–American Hispanic</td>
<td>13 (22.4)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity Hispanic</td>
<td>3 (5.2)</td>
<td></td>
</tr>
<tr>
<td>Etiology of ESRD Type 2 diabetes mellitus</td>
<td>25 (43.1)</td>
<td></td>
</tr>
<tr>
<td>Type 1 diabetes mellitus</td>
<td>4 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>19 (32.8)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>4 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>6 (10.3)</td>
<td></td>
</tr>
<tr>
<td>Total carnitine (μmol/L)</td>
<td>35.6 ± 11.5</td>
<td></td>
</tr>
<tr>
<td>Free carnitine (μmol/L)</td>
<td>20.1 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>Acylcarnitine (μmol/L)</td>
<td>15.8 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Subjective global assessment score</td>
<td>5.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Six-minute walk (metres)</td>
<td>145.0 ± 78.5</td>
<td></td>
</tr>
<tr>
<td>Sit-to-stand (count)</td>
<td>5.0 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>16.1 ± 20.6</td>
<td></td>
</tr>
<tr>
<td>ESA dose (U)</td>
<td>6417 ± 7780</td>
<td></td>
</tr>
</tbody>
</table>

*Data presented are either mean ± SD or n (%); ESRD, End Stage Renal Disease.

The location of the x-intercepts is also of interest because oxidation of fatty acids is divided between two organelles: the peroxisome and the mitochondria. The peroxisomes preferentially oxidize longer chain fatty acids, while the mitochondria have higher specificity for shorter chains [28]. Fatty acids with lengths between 10 and 16 carbons can be oxidized in either organelle. Interestingly, four of the five observed x-intercepts fell into this overlap range. This suggests that the dysfunction in fatty acid oxidation may be related to the division of labor between the peroxisomes and the mitochondria.

It is possible that longer acyl moieties may be more difficult to dialyze than the shorter acylcarnitine moieties.

**Table 2.** Partial correlation coefficients of selected acylcarnitine species

<table>
<thead>
<tr>
<th>Acylcarnitine acyl chain (chain length)</th>
<th>Sit-to-stand (<em>n</em> = 54)</th>
<th>Six-minute walk (<em>n</em> = 39)</th>
<th>SF-36 PCS (<em>n</em> = 56)</th>
<th>C-reactive protein (<em>n</em> = 46)</th>
<th>ESA dose (<em>n</em> = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid (2)</td>
<td>0.122</td>
<td>0.230</td>
<td>0.283*</td>
<td>0.240</td>
<td>0.028</td>
</tr>
<tr>
<td>Lauric acid (12)</td>
<td>−0.312*</td>
<td>−0.204</td>
<td>−0.348*</td>
<td>0.225</td>
<td>−0.083</td>
</tr>
<tr>
<td>Myristic acid (14)</td>
<td>−0.230</td>
<td>−0.041</td>
<td>−0.444*</td>
<td>0.060</td>
<td>−0.094</td>
</tr>
<tr>
<td>Palmitic acid (16)</td>
<td>−0.268</td>
<td>−0.166</td>
<td>−0.549*</td>
<td>0.125</td>
<td>0.059</td>
</tr>
<tr>
<td>Palmitoleic acid (16)</td>
<td>−0.362*</td>
<td>−0.328</td>
<td>−0.535*</td>
<td>0.145</td>
<td>−0.125</td>
</tr>
<tr>
<td>Stearic acid (18)</td>
<td>−0.163</td>
<td>−0.159</td>
<td>−0.283*</td>
<td>−0.106</td>
<td>0.028</td>
</tr>
<tr>
<td>Oleic acid (18)</td>
<td>−0.373*</td>
<td>−0.153</td>
<td>−0.606*</td>
<td>0.061</td>
<td>−0.022</td>
</tr>
<tr>
<td>Linoleic acid (18)</td>
<td>−0.237</td>
<td>−0.300</td>
<td>−0.386*</td>
<td>0.179</td>
<td>0.017</td>
</tr>
</tbody>
</table>

*Partial correlations controlled for age, gender, hemoglobin and subjective global assessment score.

*For familiarity, acyl chains are named according to the common name of the fatty acid that would be formed by the hydrolysis of the acylcarnitine ester.

*Count of maneuvers performed in 30 s.

*Metres walked in 6 min.

*Medical Outcomes Trust SF-36 PCS.

*ESA dose. *P* < 0.05.

**Table 3.** Linear regressions between acylcarnitine chain length and strength of relationship to outcome measures (*n* = 27)

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>β</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sit-to-stand</td>
<td>−0.576</td>
<td>0.332</td>
<td>0.002</td>
</tr>
<tr>
<td>Six-minute walk</td>
<td>−0.589</td>
<td>0.347</td>
<td>0.001</td>
</tr>
<tr>
<td>SF-36 PCS</td>
<td>−0.797</td>
<td>0.635</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.415</td>
<td>0.172</td>
<td>0.031</td>
</tr>
<tr>
<td>ESA dose</td>
<td>0.425</td>
<td>0.181</td>
<td>0.027</td>
</tr>
</tbody>
</table>

*Count of maneuvers performed in 30 s.

*Metres walked in 6 min.

*Medical Outcomes Trust SF-36 PCS.

*ESA dose.

**Table 4.** Trend line equations for the relationships between acylcarnitine chain length and outcome measures (*n* = 27)

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Equation</th>
<th>x-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sit-to-stand</td>
<td>y = −0.017x + 0.040</td>
<td>2.4</td>
</tr>
<tr>
<td>Six-minute walk</td>
<td>y = −0.019x + 0.150</td>
<td>7.9</td>
</tr>
<tr>
<td>SF-36 PCS</td>
<td>y = −0.038x + 0.251</td>
<td>6.6</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>y = 0.010x − 0.082</td>
<td>8.2</td>
</tr>
<tr>
<td>ESA dose</td>
<td>y = 0.008x − 0.108</td>
<td>13.5</td>
</tr>
</tbody>
</table>

*Count of maneuvers performed in 30 s.

*Metres walked in 6 min.

*Medical Outcomes Trust SF-36 PCS.

*ESA dose.
due to their higher molecular weight and lipophilicity [29].
The literature reporting reduced removal of acylcarnitines, specifically those with chain lengths greater than eight carbons, was correlative, similar to our data. We are unaware of the direct measurement of acylcarnitine removal by the dialysis process. Therefore, to confirm the exact reason behind elevated acylcarnitine moieties, further studies need to be conducted where direct measurement of both fatty acid oxidation and plasma acylcarnitine removal by dialysis is done. In order to address the possibility of dialysis affinity differentials affecting a shift in plasma acylcarnitine profiles for long-term hemodialysis patients, statistical analyses not described in this paper were performed including the subjects’ total number of months receiving dialysis treatment. No significant relationships existed between dialysis duration and any of our outcomes, acylcarnitine species or acyl chain length. Additionally, controlling for dialysis duration did not reduce the significance of the models presented in this paper.

Limitations and future directions

This was a descriptive study, and therefore our analyses can imply relationships but cannot be used to infer causation. Further experimental research will be needed to validate the hypothesis of this paper. The relationship between fatty acid oxidation dysfunction and increased red blood cell fragility was hypothesized to be related to glucose availability and proteolysis, but no blood glucose, hemoglobin A1c or leucine kinetics data were available for analysis to confirm this intermediate step in the hypothesis. In addition to glucose and leucine dynamics, future studies could also confirm dysfunctional fatty acid oxidation in dialysis patients by measuring respiratory quotients. Lastly, metabolic studies of dialysis patients are needed to directly confirm disordered fatty acid metabolism and investigate its cause.

It may also be noteworthy that the plot of acyl chain length versus correlation coefficient for ESA dose (Figure 1e) appears to contain two separate trends, suggesting that the actual relationship involves additional factors. This may be related to the above-mentioned intermediate steps in the hypothesis that were not considered due to lack of available data; our use of absolute ESA dose rather than erythropoietin resistance index or other, as yet unidentified, factors.

Conclusion

Regression results revealed the crucial relationship of acyl chain length to outcome measures: longer chains are negatively associated with physical function and health-related quality of life and are positively associated with inflammation and anemia. This data supports our hypothesis that the cause of increased plasma acylcarnitines is incomplete fatty acid oxidation and this underlying disturbance of metabolism reduces muscle function, resulting in decreased ability to function, and decreased glucose availability, resulting in worsened anemia. However, further clinical research is needed to confirm or deny the findings of this descriptive study.

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Conflict of interest statement. None declared.

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