Title: Association between resistance to erythropoiesis-stimulating agents and carnitine profile in patients on maintenance hemodialysis

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nep.13079

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Abstract

***Objective*:** Patients on dialysis are in a chronic carnitine-deficient state. This condition may be associated with abnormalities in fatty acid and organic acid metabolism; however, the details are unknown. We investigated the association between carnitine profiles before and after dialysis and the erythropoiesis-stimulating agent (ESA) resistance index (ERI), which is a significant prognostic factor in patients on maintenance hemodialysis.

***Design and Methods*:** cross-sectional study. We measured the carnitine profile of 79 patients on maintenance hemodialysis before and after dialysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The associations between the ERI and pre-dialysis carnitine profile, removal rate of various carnitines, and previously-reported ERI-related factors were investigated. Significant factors were determined with stepwise multiple regression analysis and validated with the bootstrap method. SPSS version 22.0 was used for analysis, and $P<0.05$ was considered statistically significant.

***Results*:** The removal rate of long-chain acylcarnitine with dialysis was lower than that of short-chain or medium-chain acylcarnitines. Stepwise multiple regression analysis (n=79) demonstrated that 3-hydroxy isovalerylcarnitine (C5-OH, $P<0.001$, $\beta= -0.469$) and stearoylcarnitine (C18, $P<0.001$, $\beta= 0.390$) were independent significant factors ($R^2= 0.239$) of ERI. The bootstrap method similarly indicated these two to be significant factors.

***Conclusion*:** ERI positively correlated with long-chain C18 acylcarnitine and negatively correlated with short-chain C5-OH acylcarnitine. C5-OH and C18 acylcarnitines at baseline might be contributing factors in distinguishing responders from nonresponders after L-carnitine administration.

SUMMARY AT A GLANCE

This study showed that short-chain C5-OH acylcarnitine and long-chain C18 acylcarnitine were associated with erythropoiesis-stimulating agent resistance in hemodialysis patients. These results show new important aspects regarding anemia in hemodialysis patients.

Keywords: acylcarnitine profile; hemodialysis; anemia; metabolism; removal rate

Introduction

The majority of patients on maintenance hemodialysis (HD) have comorbid anemia, and anemia is known to exacerbate prognosis and decrease quality of life (QOL).[1,2] In addition to decreased erythropoietin production in the kidney, there are many other factors that induce anemia in patients on dialysis, and it is considered that L-carnitine (LC) deficiency may be one such factor.[3] Patients on hemodialysis become LC deficient through its removal with dialysis, restricted diets, or decreased LC biosynthesis due to decreased renal function.[4]

The functions of LC include transporting long-chain fatty acids that are taken up from the blood into the cytoplasm to the mitochondria, adjusting the mitochondrial acyl-coenzyme A (CoA):CoA ratio, and eliminating harmful long-chain fatty acids. Acyl-CoA accumulates in the mitochondria with abnormalities in organic acid or fatty acid metabolism, and LC is known to play a key role in fatty acid and organic acid metabolism.[5] Despite the absence of mitochondria in mammalian red blood cells (RBC), evidence for a role of LC in RBC metabolism is suggested by the presence of LC in RBC.[6,7]

In the present study, we hypothesized that disorders of organic acid and fatty acid metabolism in patients on chronic maintenance dialysis who are in an LC deficient state are associated with the erythropoiesis-stimulating agent (ESA) resistance index (ERI).

No studies have been reported that investigated the association of ERI with carnitine and clinical data taking into account the measurements of carnitine profile before and after dialysis. We therefore measured the carnitine profile before and after dialysis in patients on chronic hemodialysis, and investigated the association between the ERI and the carnitine profile.

Subjects and Methods

Subjects

We investigated the 122 patients undergoing maintenance dialysis three times per week at our hospital, excluding those who were receiving LC, were using antibiotics for acute inflammation, had undergone artificial valve replacement, had a history of hepatitis virus infection or hepatic cirrhosis, were undergoing or had a history of cancer treatment, or had received a blood transfusion within the previous 6 months.

All the patients received intravenous iron supplementation and erythropoiesis-stimulating agent. They were all treated in accordance with the 2008 JSDT Guideline for Renal Anemia in Chronic Kidney Disease[8]. All the subjects underwent hemodialysis or hemodiafiltration via a high-performance membrane with a β2-microglobulin (β2MG) clearance of more than 50 mL/min. The median size of the membranes used was 2.1 m^2, and the interquartile range (IQR) was [1.8, 2.1]. Written informed consent was obtained from each of the study subjects. The protocol of this study was approved by the ethics committee of Tokyo Women's Medical University, and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Definition of ERI

We defined the ERI as the average weekly dose of recombinant human erythropoietin (rHuEPO) over the prior 8 weeks (IU)/ post-dialysis body weight (kg) / hemoglobin (Hb) (g/dL). A darbepoetin α (DA): rHuEPO ratio of 200:1 was used for dose conversion of rHuEPO.[9]

Laboratory measurements

For laboratory measurements, in addition to routine blood analysis for clinical purposes, the carnitine profile was measured before and after dialysis.

Blood was drawn just before week beginning HD sessions for the determination of hemoglobin, total protein (TP), albumin, blood urea nitrogen (BUN), creatinine, uric acid, potassium, calcium, phosphate, magnesium, ferritin, iron, total iron binding capacity (TIBC), C-reactive protein (CRP), whole parathyroid hormone (wPTH), bone-specific alkaline phosphatase (BSAP) and β2MG, and after HD sessions for the determinations of total protein and BUN.

The conventional urea kinetic measure known as Kt/V (single pool) was used to estimate the dialysis dose.[10]

The transferrin saturation (TSAT) was calculated using iron and TIBC measurements.

Mass spectrometry measurements

Serum samples were separated immediately after the blood collections in a refrigerated centrifuge and stored at -80 °C until analysis and were processed by the non-derivatization method and analyzed via tandem mass spectrometry (TQD, Waters Corp., Milford, MA, USA) to determine the moieties of acylcarnitines.

We measured free carnitine:C0, Acetylcarnitine:C2, Propionylcarnitine:C3, Isobutyrylcarnitine:C4, Isovalerylcarnitine:C5, 3-hydroxyisovalerylcarnitine:C5-OH, Tiglylcarnitine:C5:1, Glutarylcarnitine:C5DC, Hexanoylcarnitine:C6, Octanoylcarnitine:C8, Decanoylcarnitine:C10, Decenoylcarnitine:C10:1, Dodecanoylcarnitine:C12, Tetradecanoylcarnitine:C14, Tetradecenoylcarnitine:C14:1, Palmitoylcarnitine:C16, 3-hydroxypalmitoylcarnitine:C16OH, stearoylcarnitine:C18, 3-hydroxyoctadecenoylcarnitine:C18:1-OH, and Octadecenoylcarnitine:C18:1.

Very-long- chain acylcarnitines (chain length > 18) were not analyzed in this study. We measured each variable twice and used the average value.

We defined short-chain acylcarnitine as the species of all acylcarnitine from C2 to C5, middle-chain acylcarnitine as the species of all acylcarnitine from C6 to C12, and long-chain acylcarnitine as the species of all acylcarnitine from C14 to C18.

Because of the increased molecular weight and lipophilicity that accompanies increased chain length,[11,12] we computed the extraction ratio of long-chain aylcarnitine in consideration of blood concentration using the TP value after dialysis.

Anthropometric data

The subjects' age, sex, post-dialysis weight, etiology of end-stage renal disease (ESRD), dialysis vintage, comorbidities, and ESA (human recombinant erythropoietin) dose were collected from the subjects' medical records.

Statistical methods

Data are presented as mean ± standard deviation (SD) for normally distributed data, and as medians and interquartile range (IQR) for non-normally distributed data.

Univariate and forward-backward stepwise multivariate linear regressions were performed, with explanatory variables of age, dialysis vintage, dummy variable diabetes states (yes=1, no=0), dummy variable for angiotensive-converting-enzyme inhibitors (ACEI)/angiotensin II receptor blocker (ARB) medication (yes=1, no=0), TSAT, ferritin, spKt/V, β2MG , serum albumin, CRP, whole PTH, and carnitine profile to analyze the possible factors for ERI.[11,12,13]

Residuals for the multivariable regression were checked for normality using the Shapiro–Wilk test. To confirm the stability of our model, we used the standard SPSS bootstrap method

with 2000 bootstrap samples with 95% confidence interval. Values of $P<0.05$ were considered statistically significant. All statistical analyses were performed with IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Of the 122 patients who were undergoing maintenance HD three times per week at our hospital, six were receiving LC, two had findings of acute inflammation, three had undergone artificial valve replacement, 17 had a history of hepatitis virus (HBV, HCV) infection, and 19 were presently undergoing or had a history of cancer treatment. None of the 122 patients had a history of blood transfusion within the previous 6 months. With 4 patients having overlapping exclusion criteria, 79 patients were ultimately selected for the study.

The clinical background of each of the selected 79 patients is shown in Table 1. The mean age of the study population was 58.8±14.7 years, and 59% of the patients were men. None of the patients had an inborn error of organic acid metabolism or an inborn error of fatty acid metabolism. Although the mean TSAT was 22.2%, the median ferritin level was relatively low at 44 ng/mL.

Twenty types of carnitine profiles were measured. Very-long-chain acylcarnitines with carbon numbers greater than 18 were not measured. The pre-dialysis carnitine profile and carnitine removal rate are shown in Table 2. The removal rate was calculated with total protein correction in order to account for the impact of increased concentration due to water removal. The removal rate showed a tendency to decline with increasing carbon number in acylcarnitine.

Table 3 shows the results of the univariate regression analysis between ERI and previously reported factors associated with ERI, pre-dialysis carnitine profile, and carnitine removal rate,

and bootstrap results. In the present study population, TSAT was a factor that was significantly associated with ERI. Perhaps due to a narrow distribution of values, none of the other factors showed statistically significant associations.

Regarding the pre-dialysis carnitine profile, C5-OH and C5DC were significantly and negatively correlated with ERI, and C18 and C18:1 were significantly and positively correlated with ERI. Regarding the removal rate, none of the carnitine profiles demonstrated a significant association with ERI.

Table 4 provides the results from the stepwise multiple regression analysis. The C18 level and C5-OH level were determined to be significant factors of ERI, with the former having a positive correlation and the latter having a negative correlation. To verify the reliability of the selected factors, bootstrapping multiple regression analysis of C18 and C5-OH was conducted with the brute force approach. The results from this analysis similarly showed that both factors were significant factors.

Discussion

The present study revealed that ERI is positively correlated with long-chain C18 acylcarnitine and is negatively correlated with short-chain C5-OH acylcarnitine. The mean TSAT in the subject population in this study was 22.2%, but the median serum ferritin was relatively low at 44 ng/ml. Ferritin was measured by electro-chemiluminescence immunoassay, which gives higher values than other measurement methods.[14] Thus, it is possible that TSAT and ferritin would not be selected statistically as significant factors in subject populations that tend to have little stored iron or when distributions of TSAT and ferritin are narrow, and that C5-OH acylcarnitine and C18 acylcarnitine would be identified as significant factors.

In addition to iron deficiency, possible factors of ESA resistance also include gastrointestinal bleeding, infection, cancer, hemolysis due to prosthetic heart valves, the use

of RAS inhibitors, inadequate dialysis, and severe hyperparathyroidism.[8,13,14)] These factors have a major effect on responsivity to ESA. Patients who had undergone blood transfusion within the previous 6 months and those with acute infection or cancer, those who had undergone valve replacement, and those with a history of viral hepatitis or cirrhosis of the liver were therefore excluded from the study.

The carnitine pathway is essential for long-chain fatty acid uptake from the cytoplasm into the mitochondria. When there is a disruption in this pathway, long-chain fatty acids cannot undergo β-oxidation in the mitochondria. Moreover, organic acids and long-chain fatty acids that have accumulated in the mitochondria are cytotoxic, and free carnitine is required to remove them from the mitochondria.[5]

Although erythrocytes do not contain mitochondria, the actions of L-carnitine during erythrocyte metabolism are believed to include repairing the erythrocyte cell membrane after hyperoxidation, strengthening the lining protein spectrin and improving the packing of phospholipids, and supporting the process of erythroblast formation by inhibiting caspase 3, the apoptosis of mature erythrocytes during hematopoiesis, and the phagocytosis of erythroblasts by macrophages by means of phosphatidylserine expression.[16)] Valssopoulos et al. also found that L-carnitine improves erythrocyte deformability, which is diminished in dialysis patients, and induces an increase in Ret expression level.[17)]

ERI demonstrated an inverse correlation with C5-OH, which is correlated with disorders of organic acid metabolism. However, it is unknown whether this finding signifies an abnormality in organic acid metabolism, a problem with the substrate, a hindrance in enzymatic activations, or an accumulation of deoxyribonucleic acid (DNA) damage,[16] and whether there was a phenomenon resembling RNA interference due to uremic toxins. A

mechanism from the perspective of disorders of organic acid metabolism is unknown from the present study.

From the perspective of mass transfer, the following possibility was considered. Although approximately 75% of C5-OH was removed through dialysis, C5-OH accumulated in erythrocytes does not readily travel extracellularly. For this reason, its removal is difficult and its transport to the extracellular space requires a long period of time. In other words, C5-OH may be reflecting erythrocyte survival, and consequently, C5-OH may be indicating a correlation between ERI and erythrocyte survival.

In tandem mass spectrometry, there are newborn cutoff values for acylcarnitine profiles; however, cutoff values and a standard range of acylcarnitine profiles have not been reported in adults or in patients on dialysis. Although newborn cutoff values may be used in healthy adults who grew up without being noted for having an inborn error of metabolism, since free carnitine is insufficient and low in patients on dialysis, the overall level of acylcarnitine, which is a fatty acid bound to free carnitine, may also be deficient and low. It is unclear through the present study alone whether C5-OH and C18 levels in patients on dialysis might be abnormal compared with the standard levels in patients on dialysis. We demonstrated that C5-OH observed in abnormalities of organic acid metabolism and C18 observed in abnormalities of fatty acid metabolism were selected as significant factors, and these findings indicated the presence of associations between these abnormalities and ERI in patients on dialysis. However, it is unknown whether or not these disorders have a causal relationship with ERI.

In a meta-analysis of double-blind placebo-controlled studies of LC replacement therapy to treat anemia in patients on chronic maintenance dialysis, it was reported that this replacement therapy is effective in improving anemia and ERI.[17] However, both effective and ineffective cases exist with LC administration, and it is unknown which factor causes this. To

predict for which patients LC administration would be effective and to reduce unnecessary administration, prediction of the effect before commencing replacement therapy is necessary; the standard levels of C18 and C5-OH immediately before hemodialysis that were determined by the present study may be useful for such differentiation.

Previous studies have been conducted that investigated ERI and the acylcarnitine profile in patients on hemodialysis. These studies found that there is a statistically significant association between ERI and acylcarnitine.[7] However, it could not be shown which particular acylcarnitine is associated with ERI.[20]

In the present study, we conducted the analysis including not only the acylcarnitine profile, but also the acylcarnitine removal rate and various factors related to ERI. As a result, long-chain acylcarnitine (C18) was identified as a significant factor with a positive correlation; moreover, C5-OH acylcarnitine was also determined to be a significant factor but with a negative correlation.

In addition, it has also been reported that long-chain acylcarnitine is a predictor of one-year cardiovascular mortality in patients on dialysis,[21] indicating that abnormalities in organic acid and fatty acid metabolism are crucial in patients on dialysis. Further studies on this subject are necessary.

Next, we calculated the removal rate of long-chain acylcarnitine, adjusting for total protein levels before and after dialysis. We found that, similarly to previous reports, the removal rate of long-chain acylcarnitine was lower than that of short-chain or medium-chain acylcarnitines,[11, 12, 22] The reason for this is considered to be because long-chain acylcarnitines have greater molecular weight and lipophilicity. However, in all patients in the present study, hemodialysis or hemodiafiltration using a high performance membrane with high β2MG (molecular weight: 11800) clearance was used. Since the molecular weight of long-chain acylcarnitines is around 500, the lipophilic nature of long-chain acylcarnitine was

thought to be the primary reason for its low removal rate. Current dialysis methods are considered to have poor removal rates of long-chain acylcarnitine; therefore, if removal is required, an approach different from dialysis must be considered. As a potential method, removal may be feasible by enhancing the efficiency of fatty acid metabolism via LC administration or peroxisome activation.

Associations between the removal rates of various carnitines and ERI were not observed. These findings indicated that, in the clinical setting, measurement of the carnitine profile after dialysis to determine the association with ERI may not be necessary.

ERI positively correlated with long-chain C18 acylcarnitine and negatively correlated with short-chain C5-OH acylcarnitine. C5-OH and C18 acylcarnitines at baseline might be contributing factors in distinguishing responders from non-responders after LC administration.

The present study had several limitations. First, we could not exclude the potential effect of other unknown confounders. Second, this study was based on a retrospective observational design, and the sample size was small because of being a single-center cohort study. We confirmed the reliability of explanatory variables selected by stepwise analysis using the bootstrap method; however, further investigation including a greater number of cases from multiple centers is necessary. Third, the amount of LC and fatty acid composition in the meal consumed by the study patients before blood collection was not evaluated. It has been reported that the bioavailability of LC is less than 15% with its oral administration in healthy individuals.[23] It is therefore presumed that the bioavailability is similarly low in patients on chronic maintenance dialysis. Nonetheless, meal analysis is necessary to confirm this when conducting a similar study. Fourth, as Chait et al. reported, as an index, ERI is similar to weight- and non-weight-adjusted EPO.[24)] In the existing guidelines, ERI is not identified as associated with prognosis. ERI should be defined in terms of the results of prospective trials

in which its association with prognosis was evaluated. Finally, it is necessary to determine whether the association observed between the carnitine profile and ERI in the present study was a correlation between the two factors or was a causal relationship. A large-scale randomized controlled study should be conducted to verify the association between ERI and changes in the carnitine profile due to LC administration.

Conclusions

ERI positively correlated with long-chain C18 acylcarnitine and negatively correlated with short-chain C5-OH acylcarnitine. The removal rate of long-chain acylcarnitine with dialysis is low. Further future investigation is necessary.

References

1. Pisoni RL, Bragg-Gresham JL, Young EW, et al. Anemia management and outcomes from 12 countries in the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am J Kidney Dis.*2004;44(1):94-111.
2. Leaf DE, Goldfarb DS. Interpretation and review of health-related quality of life data in CKD patients receiving treatment for anemia. *Kidney Int.* 2009;75(1):15-24.
3. Labonia WD. L-carnitine effects on anemia in hemodialyzed patients treated with erythropoietin. *Am J Kidney Dis.*1995;26(5):757-64.
4. Fornasini G1, Upton RN, Evans AM. A pharmacokinetic model for L-carnitine in patients receiving haemodialysis. *Br J Clin Pharmacol.* 2007;64(3):335-45.
5. Vaz FM, Wanders RJ. Carnitine biosynthesis in mammals. *Biochem J.* 2002;1;361(Pt 3):417-29.
6. Kudoh Y, Aoyama S, Torii T, et al. Long-term effects of oral L-carnitine supplementation on anemia in chronic hemodialysis. Cardiorenal Med. 2004;Apr;4(1):53-9. doi: 10.1159/000360865.
7. Reuter SE, Faull RJ, Ranieri E, Evans AM. Endogenous plasma carnitine pool composition and response to erythropoietin treatment in chronic haemodialysis patients. Nephrol Dial Transplant. 2009;Mar;24(3):990-6.
8. 2008 Japanese Society for Dialysis Therapy: Guidelines for Renal Anemia in Chronic Kidney Disease. Therapeutic Apheresis and Dialysis 2010;14(3):240-275
9. Aljama P, Bommer J, Canaud B, et al. Practical guidelines for the use of NESP in treating renal anaemia. *Nephrol Dial Transplant.* 2001;16 Suppl 3:22-8.
10. Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: An analysis of error. *J Am Soc Nephrol* 1993;14; 1205-1213

11. Lin YS, Yang WC, Chen TW, Lin CC. Changes in the composition of carnitines in hemodialysis patients. *Kidney Int.* 2005;68(3):1368; author reply 1368-9.
12. Csiky B, Bene J, Wittmann I, Sulyok E, Melegh B. Effect of hemodialysis session on the dynamics of carnitine ester profile changes in L-carnitine pretreated end-stage renal disease patients. *Int Urol Nephrol.* 2013 Jun;45(3):847-55.
13. KDIGO Clinical Practice Guideline for Anemia in CKD. *Kidney Int.* Suppl. 2012;2 : 279-335
14. Locatelli F, Aljama P, Bárány P, et al. Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure. *Nephrol Dial Transplant.* 2004;19 Suppl 2:ii1-47.
15. Daigo Kamei, Ken Tsuchiya, Hitomi Miura, Kosaku Nitta, Takashi Akiba. Inter-method variability of ferritin and transferrin saturation measurement methods in patients on hemodialysis. *Therapeutic Apheresis and Dialysis.* 2017 Feb;21(1):43-51.
16. Bonomini M, Zammit V, Pusey CD, De Vecchi A, Arduini A. Pharmacological use of L-carnitine in uremic anemia: has its full potential been exploited? Pharmacol Res. 2011 Mar;63(3):157-64.
17. Vlassopoulos DA, Hadjiyannakos DK, Anogiatis AG, et al. Carnitine action on red blood cell osmotic resistance in hemodialysis patients. J Nephrol. 2002 Jan-Feb;15(1):68-73.
18. Rao M, Li L, Demello C, et al.Mitochondrial DNA injury and mortality in hemodialysis patients. *J Am Soc Nephrol.* 2009;20:189–196.
19. Hurot JM, Cucherat M, Haugh M, Fouque D. Effects of L-carnitine supplementation in maintenance hemodialysis patients: a systematic review. *J Am Soc Nephrol.* 2002;13(3):708-14.

20. Murphy WJ1, Steiber A, Connery GC, Carder J, Spry L, Hoppel C. Altered carnitine metabolism in dialysis patients with reduced physical function may be due to dysfunctional fatty acid oxidation. *Nephrol Dial Transplant.* 2012;27(1):304-10.

21. Kalim S, Clish CB, Wenger J, et al. A plasma long-chain acylcarnitine predicts cardiovascular mortality in incident dialysis patients. *J Am Heart Assoc.* 2013;5;2(6):e000542. doi: 10.1161/JAHA.113.000542.

22. Sirolli V, Rossi C, Di Castelnuovo A, et al.Toward personalized hemodialysis by low molecular weight amino-containing compounds: future perspective of patient metabolic fingerprint. *Blood Transfus.* 2012;10 Suppl 2:s78-88.

23. Evans A. Dialysis-related carnitine disorder and levocarnitine pharmacology. *Am J Kidney Dis.* 2003;41(4 Suppl 4):S13-26.

24. Chait Y, Kalim S, Horowitz J, et al. The greatly misunderstood erythropoietin resistance index and the case for a new responsiveness measure. *Hemodial Int.* 2016 Jul;20(3):392-8.

Table 1. Baseline characteristics of enrolled patients (n=79)

Variable	Mean ± SD or median [IQR]	N
age (year)	58.8 ± 14.7	
Gender (Male/Female)		47/32
Etiology of ESRD		
Glomerulonephritis		45
Type 2 diabetes mellitus		16
Hypertension		8
Hereditary disease		5
Graft loss		3
the others		2
Dialysis vintage (year)	7 [2, 16]	
HD（High performance membrane）/ HDF		70/9
Dry weight (DW)	54.2 [45.3, 61.5]	
Hemoglobin (g/dL)	11.1±1.1	
Total protein (g/dL)	6.7±0.4	
Albumin (g/dL)	3.7 [3.5, 3.9]	
BUN (mg/dL)	62.4±14.6	
Creatinine (mg/dL)	11.1±2.5	
Uremic acid (mg/dL)	7.6±1.2	
Potassium (mEq/L)	5.0±0.7	
Total Calcium (mg/dL)	8.8 [8.5, 9.4]	
Phosphate (mg/dL)	5.2 [4.4, 5.9]	
Magnesium (mg/dL)	2.0 [1.9, 2.2]	
Iron (μg/dL)	59 [38, 78]	
TIBC (μg/dL)	270±45	
Transferrin saturation: TSAT (%)	22.2 [14.1, 29.1]	
Ferritin (ng/mL)	44 [23, 90]	
CRP (mg/dL)	0.11 [0.06, 0.30]	
HbA1c (%)	5.2 [4.95, 5.60]	
spKt/V un	1.44 [1.28, 1.64]	
whole PTH (pg/mL)	96.6 [50.5, 153.5]	
BSAP (μg/L)	12.4 [10.2, 19.4]	
β2-microglobulin (mg/L)	27.3±5.8	
ESA doses/week (IU/week)	4000 [2000, 6000]	
ESA doses/DW/week (IU/kg/week)	84.7 [39.1, 133]	
ESA doses/Hb/week (IU/(g/dL)/week)	391 [182, 571]	
ESA doses/kg/(g/dL)/week: ERI	8.0 [3.7, 12.8]	

footnote: HD, hemodialysis; HDF, hemodiafiltration; CPR, C-reactive protein; whole PTH, whole parathyroid hormone; BSAP, bone-specific alkaline phosphatase; ESA, erythropoiesis-stimulating agent.

Table 2. Value and removal rate of each carnitine moiety (n=79)

	Value before HD session	Removal rate (%)
C0 (nmol/mL)	21.46 [17.59, 26.99]	72.1±8.3
Acyl/C0	0.55 [0.48, 0.66]	
C2 (nmol/mL)	8.509 [6.685, 11.355]	58.3 [43.6, 71.0]
C3 (nmol/mL)	0.334 [0.258, 0.446]	76.3 [69.7, 81.0]
C4 (nmol/mL)	0.517 [0.387, 0.667]	77.9 [72.5, 82.3]
C5 (nmol/mL)	0.247 [0.215, 0.288]	44.0±12.1
C5OH (nmol/mL)	0.183 ± 0.048	75.1 [69.4, 78.9]
C5:1 (nmol/mL)	0.054 [0.042, 0.062]	68.1±11.1
C5DC (nmol/mL)	1.036 [0.809, 1.318]	71.2 [65.1, 76.5]
C6 (nmol/mL)	0.041 [0.032, 0.055]	52.7 [39.9, 69.9]
C8 (nmol/mL)	0.188 [0.144, 0.283]	71.9 [58.3, 81.7]
C10 (nmol/mL)	0.345 [0.253, 0.510]	67.0 [52.5, 76.9]
C10:1 (nmol/mL)	0.295 [0.204, 0.406]	69.0 [57.4, 77.9]
C12 (nmol/mL)	0.086 [0.061, 0.129]	53.8 [35.5, 71.7]
C14 (nmol/mL)	0.033 [0.027, 0.043]	23.3 [−3.6, 40.1]
C14:1 (nmol/mL)	0.078 [0.054, 0.138]	28.8 [−41.7, 54.4]
C16 (nmol/mL)	0.094±0.028	4.6±24.4
C16 OH (nmol/mL)	0.005±0.001	14.9 [−1.5, 32.5]
C18 (nmol/mL)	0.036 [0.029, 0.043]	4.2±17.6
C18:1OH (nmol/mL)	0.008 [0.006, 0.010]	5.6 [−16.6, 18.2]
C18:1 (nmol/mL)	0.120 [0.084, 0.157]	−9.4 [−35.7, 16.4]

footnote: C0, free carnitine; C2,Acetylcarnitine; C3, Propionylcarnitine; C4, Isobutyrylcarnitine; C5, Isovalerylcarnitine; C5-OH, 3-hydroxyisovalerylcarnitine; C5:1, Tiglylcarnitine; C5DC, Glutarylcarnitine; C6, Hexanoylcarnitine; C8, Octanoylcarnitine; C10, Decanoylcarnitin; C10:1, Decenoylcarnitine; C12, Dodecanoylcarnitine; C14, Tetradecanoylcarnitine; C14:1, Tetradecenoylcarnitine; C16, Palmitoylcarnitine; C16OH, 3-hydroxypalmitoylcarnitine; C18, stearoylcarnitine; C18:1-OH, 3-hydroxyoctadecenoylcarnitine; C18:1 Octadecenoylcarnitine.

Table 3. Univariate regression analysis of the correlates of ERI and previously reported factors associated with ERI, pre-dialysis carnitine profile, and carnitine removal rate, and bootstrap results

				Bootstrap Result (2000 Replicas)			
	β	95%CI	*P*	Bias	S.E	95% CI	*P*
Age	−0.066	−0.148 to 0.081	0.561	0.000	0.048	−0.128 to 0.059	0.477
HD vintage	−0.062	−0.207 to 0.117	0.584	0.002	0.079	−0.204 to 0.106	0.574
diabetes states (yes=1, no=0)	−0.133	−6.784 to 1.733	0.241	−0.033	1.811	−6.072 to 1.097	0.166
ARB/ACEI Medication (yes=1, no=0)	−0.007	−3.473 to 3.273	0.953	0.049	1.679	−3.478 to 3.035	0.954
TSAT	−0.239	−33.031 to −1.377	0.034	0.025	8.623	−35.349 to −1.806	0.050
ferritin	−0.005	−0.027 to 0.026	0.962	−0.005	0.019	−0.043 to 0.026	0.972
sp Kt/Vun	0.030	−4.748 to 6.180	0.766	0.089	2.953	−4.678 to 7.024	0.811
β2MG	−0.074	−0.385 to 0.195	0.516	−0.004	0.195	−0.517 to 0.268	0.627
Alb	0.077	−3.166 to 6.444	0.499	0.173	2.167	−2.122 to 6.397	0.448
CRP	0.016	−2.155 to 2.491	0.886	0.189	1.221	−1.726 to 3.192	0.865
whole PTH	0.130	−0.007 to 0.025	0.255	0.000	0.015	−0.015 to 0.041	0.564
BSAP	0.083	−0.091 to 0.197	0.469	0.006	0.112	−0.134 to 0.312	0.620
C0	−0.103	−0.291 to 0.109	0.368	−0.019	0.114	−0.340 to 0.085	0.448
Acyl/C0	0.055	−7.409 to 12.205	0.628	0.362	4.000	−4.849 to 11.245	0.539
C2	0.022	−0.405 to 0.490	0.850	−0.029	0.207	−0.465 to 0.354	0.832
C3	−0.089	−14.808 to 6.422	0.434	−0.350	5.171	−15.647 to 4.872	0.424
C4	−0.216	−11.821 to 0.138	0.055	−0.030	3.094	−12.685 to −0.325	0.051
C5	0.021	−12.680 to 15.266	0.854	−4.353	11.254	−34.571 to 8.991	0.846
C5OH	−0.349	−87.038 to −21.072	0.002	0.151	18.004	−91.058 to −19.604	0.003
C5:1	−0.205	−165.901 to 6.580	0.070	0.002	46.492	−176.946 to 10.419	0.084
C5DC	−0.285	−8.705 to −	0.011	0.006	2.254	−9.652 to	0.038

		1.173				−0.486	
C6	−0.058	−56.635 to 33.667	0.614	5.527	30.944	−57.768 to 84.934	0.369
C8	−0.049	−8.003 to 5.159	0.668	1.787	5.543	−8.305 to 16.010	0.391
C10	−0.001	−4.679 to 4.625	0.991	1.620	3.627	−1.997 to 11.895	0.988
C10:1	−0.056	−12.853 to 7.765	0.625	0.229	4.684	−10.604 to 7.995	0.559
C12	0.081	−21.008 to 44.434	0.478	2.121	17.884	−15.596 to 57.116	0.479
C14	0.012	−128.505 to 142.540	0.918	2.667	74.219	−112.765 to 182.051	0.928
C14:1	0.069	−17.062 to 32.150	0.543	0.592	10.704	−11.490 to 30.364	0.468
C16	0.195	−7.321 to 108.785	0.086	−3.038	46.020	−31.347 to 141.768	0.325
C16 OH	0.116	−724.227 to 2261.525	0.308	−3.753	701.724	−594.879 to 2089.364	0.288
C18	0.244	14.362 to 275.538	0.030	−15.755	113.737	−65.220 to 349.228	0.260
C18:1OH	0.013	−646.842 to 723.910	0.911	19.852	284.987	−473.562 to 658.869	0.891
C18:1	0.231	1.402 to 63.069	0.041	−0.518	23.765	−7.038 to 83.570	0.193
C0 removal rate	−0.049	−24.795 to 15.982	0.668	−0.008	8.979	−22.783 to 13.072	0.624
C2 removal rate	0.005	−7.895 to 8.284	0.962	0.076	3.327	−5.790 to 7.225	0.956
C3 removal rate	0.082	−11.077 to 23.774	0.470	−1.115	7.855	−13.097 to 18.029	0.387
C4 removal rate	−0.078	−26.958 to 13.134	0.494	−0.221	10.369	−30.919 to 12.884	0.458
C5 removal rate	−0.104	−20.207 to 7.459	0.362	0.166	6.095	−17.986 to 5.606	0.319
C5OH removal rate	−0.053	−22.893 to 14.217	0.643	−0.379	8.257	−21.961 to 10.558	0.607
C5:1 removal rate	−0.059	−19.011 to 11.153	0.605	0.259	6.788	−16.221 to 9.879	0.571
C5DC removal rate	−0.198	−34.390 to 2.006	0.080	0.203	9.084	−33.762 to 2.739	0.077
C6 removal rate	0.051	−4.005 to 6.348	0.653	0.028	2.029	−2.621 to 5.377	0.565
C8 removal rate	0.152	−3.179 to 16.678	0.180	−0.026	4.834	−2.474 to 16.562	0.169
C10 removal rate	0.203	−0.633 to 14.285	0.072	0.202	3.294	0.794 to 13.829	0.035
C10:1 removal rate	0.167	−2.617 to 18.113	0.141	0.026	4.682	−1.307 to 17.122	0.104
C12 removal	0.087	−2.935 to	0.443	0.083	1.999	−1.871 to	0.336

rate		6.641				6.184	
C14 removal rate	−0.011	−5.135 to 4.646	0.921	0.047	1.958	−3.902 to 3.797	0.902
C14:1 removal rate	0.127	−0.627 to 2.251	0.265	0.018	0.638	−0.410 to 2.193	0.158
C16 removal rate	0.005	−6.733 to 7.058	0.963	0.087	3.221	−5.904 to 6.761	0.954
C16 OH removal rate	0.066	−4.882 to 8.917	0.562	0.009	2.901	−3.448 to 7.729	0.501
C18 removal rate	0.060	−7.004 to 12.067	0.599	0.122	4.457	−5.800 to 11.687	0.561
C18:1OH removal rate	0.029	−4.757 to 6.157	0.799	−0.065	2.061	−3.473 to 4.655	0.732
C18:1 removal rate	0.105	−1.832 to 5.029	0.356	−0.008	1.757	−1.633 to 5.337	0.359

footnote: HD, hemodialysis; TSAT, transferrin saturation; β2MG, β2- macroglobulin; Alb, albumin; CRP, C-reactive protein; whole PTH, whole parathyroid hormone; BSAP, bone-specific alkaline phosphatase; C0, free carnitine; C2,Acetylcarnitine; C3, Propionylcarnitine; C4, Isobutyrylcarnitine; C5, Isovalerylcarnitine; C5-OH, 3-hydroxyisovalerylcarnitine; C5:1, Tiglylcarnitine; C5DC, Glutarylcarnitine; C6, Hexanoylcarnitine; C8, Octanoylcarnitine; C10, Decanoylcarnitin; C10:1, Decenoylcarnitine; C12, Dodecanoylcarnitine; C14, Tetradecanoylcarnitine; C14:1, Tetradecenoylcarnitine; C16, Palmitoylcarnitine; C16OH, 3-hydroxypalmitoylcarnitine; C18, stearoylcarnitine; C18:1-OH, 3-hydroxyoctadecenoylcarnitine; C18:1 Octadecenoylcarnitine.

Table 4. Multiple regression analysis for the correlates of ERI by forward-backward stepwise method and bootstrap results.

	β	95%CI	*P*	Bootstrap Results (2000 Replicas)			
				Bias	S.E	95% CI	*P*
C5OH	−0.469	−112 to −40	0.001>	3.111	20.101	−107 to −31.4	0.001>
C18	0.390	108 to 354	0.001>	−18.112	105.330	21.8 to 406	0.028

ANOVA $p<0.001$; $R = 0.509$ ($R^2 = 0.259$, adjusted $R^2 = 0.239$); Durbin-Watson ratio＝2.262.

footnote: C5-OH, 3-hydroxyisovalerylcarnitine; C18, stearoylcarnitine.