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Impact of Circulating N-Acylethanolamine Levels with Clinical and Laboratory Endpoints in Hemodialysis Patients

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Abstract

Background: Patients with end-stage renal disease (ESRD) on maintenance hemodialysis (MHD) are particularly susceptible to dysregulation of energy metabolism, which may manifest as protein energy wasting and cachexia. In recent years, the endocannabinoid system (ECS) has been shown to play an important role in energy metabolism with potential relevance in ESRD. *N*-acylethanolamines (NAEs) are a class of fatty acid amides which include the major endocannabinoid ligand, anandamide, and the endogenous peroxisome proliferator-activated receptor-α (PPAR-α) agonists, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA).

Methods: Serum concentrations of OEA and PEA were measured in MHD patients and their correlations with various clinical/laboratory indices were examined. Secondarily, we evaluated the association of circulating PEA and OEA levels with 12-month all-cause mortality.

Results: Both serum OEA and PEA levels positively correlated with HDL-C levels and negatively correlated with body fat and body anthropometric measures. Serum OEA levels correlated positively with serum interleukin-6 (IL-6) (rho=0.19, p=0.004). Serum PEA and IL-6 showed a similar but nonsignificant trend (rho= 0.12, p=0.07). Restricted cubic spline analyses showed that increasing serum OEA and PEA both trended towards higher mortality risk, and these

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Conflict of Interest Statement: KKZ, HM and DP declare the following conflict of interest: they are inventors in a patent application filed by the University of California, Irvine, which protects certain aspects of the work described in the present article. The remaining authors have nothing to disclose.

associations were statistically significant for PEA (PEA≥4.7 pmol/mL, reference: PEA<4.7 pmol/mL) after adjustments in a Cox model (hazard ratio [HR] 2.99, 95% CI 1.04, 8.64).

Conclusions: In MHD patients, OEA and PEA are significantly correlated with variables related to lipid metabolism and body mass. Additionally, higher serum levels of PEA are associated with mortality risk. Future studies are needed to examine the potential mechanisms responsible for these findings and their clinical implications.

Keywords

End stage renal disease; maintenance hemodialysis; mortality; endocannabinoid system; oleoylethanolamide; palmitoylethanolamide

INTRODUCTION

The population of individuals with advanced chronic kidney disease (CKD) and end-stage renal disease (ESRD) requiring maintenance hemodialysis (MHD) is on a steady rise [1, 2]. While in the general population obesity and higher BMI are associated with increased risk of mortality [3, 4], the opposite has been shown in certain patient populations, including in chronic obstructive pulmonary disease (COPD), acquired immunodeficiency syndrome, and chronic heart failure [5–7] . This phenomenon has been termed the “obesity paradox” and has also been well-established in ESRD patients, in whom higher BMI is associated with reduced risk of cachexia and death [8, 9].

The endocannabinoid system (ECS) consists of a set of lipid-derived ligands and their receptors that are involved in the regulation of metabolism and energy homeostasis [10–12]. The two most extensively studied endocannabinoids (ECs)—2-arachidonoyl-*sn*-glycerol (2-AG) and arachidonoylethanolamide (AEA, anandamide)—primarily exert their effects via the canonical cannabinoid receptors 1 and 2 (CB_1R and CB_2R). In a cohort of patients with ESRD undergoing MHD therapy, we recently showed that circulating 2-AG and AEA levels correlate with markers of body mass and risk of mortality [13, 14]. For example, we found that increased serum 2-AG levels were associated with higher serum triglycerides, lower serum high density lipoprotein (HDL) levels, and higher indices of body mass using anthropometric measures. In addition, we found that higher serum 2-AG levels were associated with decreased risk of mortality in patients on MHD. Interestingly, serum AEA concentrations did not demonstrate the same correlations as 2-AG and in some analyses were found to have the opposite associations. These findings are intriguing given that both AEA and 2-AG are considered key major ligands of the endocannabinoid system with activity at the CB receptors.

Given these discoveries, we sought to determine whether circulating levels of other *N*-acylethanolamines (NAEs) that share biosynthetic and degradative enzymes with the endocannabinoid AEA exhibit similar associations. In contrast to AEA, however, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) do not bind and activate CB_1Rs and CB_2Rs. Instead, they exert analgesic and anti-inflammatory effects primarily, if not exclusively, via binding to peroxisome proliferator-activated receptor (PPAR)-α, though interactions with the G-protein coupled receptor 119 (GRP119) and the transient receptor

potential vanilloid type 1 (TRPV1) have also been reported [15–20]. The downstream effects of PPAR-α activation by OEA and PEA are of particular interest in patients undergoing MHD because this nuclear transcription factor is a critical transcriptional activator of genes involved in fatty acid oxidation and also contributes to the control of inflammation via downregulation of nuclear factor-kappa B (NF-κB) [21–23]. In this study, we set out to evaluate the association of circulating OEA and PEA levels with laboratory and clinical parameters including serum lipids and body anthropometric measures in a cohort of ESRD patients undergoing MHD.

METHODS

Study Population

For this study, our cohort consisted of patients from the prospective *Malnutrition, Diet and Racial Disparities in Chronic Kidney Disease* (MADRAD) study who received hemodialysis treatment from various outpatient clinics in Southern California. A subgroup of 235 MADRAD patients aged 18 years and older with serum OEA and PEA measurements were selected. A total of 3 patients were removed for errors or duplicate measurements. The final study cohort consisted of 232 MADRAD patients.

This study was approved by the institutional review committees of the Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA, and the University of California, Irvine Medical Center, Orange, CA. Patients were included in the study if they 1) received hemodialysis ≥4 weeks, 2) did not have a limited life expectancy <6 years (i.e., stage IV malignancy), and 3) provided written/signed informed consent.

Demographic, Clinical and Laboratory Measurements

MADRAD study coordinators collected baseline data on demographic, clinical, and body anthropometry measures, including biceps and triceps skinfolds, mid-arm circumference, mid-arm muscle circumference, waist circumference, and near infra-red total body fat percentage. Additional details about depression (Beck Depression Index II) and quality of life (Short From 36) measures have been previously reported [24].

Routine laboratory measurements closest to the blood draw dates for baseline OEA or PEA were obtained using electronic health records from the outpatient dialysis clinics. Standardized methods were used to measure pre-dialysis blood samples, typically within 24 hours from collection. Dialysis vintage for MHD patients was defined as the time between the dates of the patient's first dialysis treatment and serum OEA or PEA measurement. MADRAD study coordinators determined at the time of study entry the presence of diabetes as a pre-existing comorbidity by a combination of patient self-reported history and International Classification of Diseases-9 (ICD-9) codes using the electronic health records.

Serum Samples and Analyses

Prior to weekly dialysis sessions, patient serum was extracted and stored frozen at −80°C. ELISA assay kits from research and development (R&D) systems based in Minneapolis,

MN, and from Affymetric ThermoFisher Scientific were used to measure the concentration of serum interleukin (IL-6) by following the manufacturer's protocol.

Lipid extraction and FAEs and MAGs analysis

Lipid extraction and analysis were performed as previously described [25–26]. 0.5 mL of serum was placed in 1.0 mL of methanol solution containing the internal standards, [$^2H_4$]-OEA and [$^2H_4$]-PEA (Cayman Chemical, Ann Arbor, MI, USA). Lipids were extracted with chloroform (2 mL) and washed with sterile 0.9 % saline (0.5 mL). Organic phases were collected and separated by open-bed silica gel column chromatography as previously described [26]. Eluate was gently dried under ultra-high purity N_2 stream (99.998% pure) and resuspended in 0.1 mL of methanol: chloroform (9:1), with 1μL injection for ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) analysis.

Data was acquired using an Acquity I Class UPLC with in-line connection to a Xevo TQ-S Micro Triple Quadrapole Mass Spectrometer (Waters Corporation, Milford, MA, USA) with electrospray ionization (ESI) sample delivery. Lipids were separated using an Acquity UPLC BEH C_{18} column (2.1 x 50 mm i.d., 1.7 μm, Waters) and inline Acquity guard column (UPLC BEH C_{18} VanGuard PreColumn; 2.1 x 5 mm i.d.; 1.7 μm, Waters), and eluted by a gradient of water and methanol (containing 0.25% acetic acid, 5 mM ammonium acetate) at a flow rate of 0.4 mL/min and gradient: 80% methanol 0.5 min, 80% to 100% methanol 0.5 to 2.5 mins, 100% methanol 2.5 to 3.0 mins, 100% to 80% methanol 3.0 to 3.1 mins, and 80% methanol 3.1 to 4.5 mins. Column was maintained at 40°C and samples were kept at 10°C in sample manager. MS detection was in positive ion mode with capillary voltage maintained at 1.10 kV and Argon (99.998%) was used as collision gas. Lipids were quantified using a stable isotope dilution method detecting proton or sodium adducts of the molecular ions $[M + H/Na]^+$ in multiple reaction monitoring (MRM) mode. Sample processing and LCMS analyses for experiments occurred independently of other experiments. Extracted ion chromatograms for MRM transitions were used to quantify analytes: OEA (m/z = 326.4 > 62.1), PEA, (m/z = 300.3 > 62.1), [$^2H_4$]-OEA (m/z = 330.4 > 66.0), and [$^2H_4$]-PEA (m/z = 304.3 > 66.1) as internal standards. Controls included one "blank" sample that was processed and analyzed identically to samples, except no serum was added. This control revealed no detectable lipids.

Exposure and Outcome Ascertainment

In primary analysis, we examined serum levels of OEA and PEA as continuous variables and their associations with clinical and laboratory measurements.

In secondary analysis, OEA and PEA levels were categorized by dichotomization at their median values (<6.9 and ≥6.9 pmol/mL; <4.7 and ≥4.7 pmol/mL, respectively) and their association with 12-month all-cause mortality was evaluated. Follow-up time was measured starting from the blood draw date until death, transplantation, loss-to-follow up, or the end of the study period. MADRAD study coordinators collected mortality and censoring information on an annual basis and were examined by MADRAD nephrologists (C.M.R. and K.K.-Z.).

Statistical Analysis

Patient baseline characteristics were described across OEA and PEA dichotomies using means±standard deviations (SD), medians (interquartile range(IQR)), or proportions, as appropriate based on variable type and distribution. Chi-squared or Fisher Exact tests were used to examine differences in categorical variables and T-tests or Mann-Whitney U tests were used to assess differences in continuous variables across OEA and PEA dichotomies. For serum OEA and PEA, normality was assessed using the Shapiro-Wilk test and histograms to check data distribution. Spearman's rank correlation (Rho) was used to examine the correlations between serum OEA and PEA levels with clinical, laboratory, body anthropometric, and quality of life measurements.

To examine the association between serum OEA and PEA groups with all-cause mortality, Cox proportional hazards models were used. Proportional hazards assumptions were assessed using the Supremum test and graphical analysis. Associations between continuous serum OEA and PEA levels and 12-month all-cause mortality were evaluated using restricted cubic spline models. For these models, hierarchical levels of adjustment were as follows: (1) Model 1 – Unadjusted; (2) Model 2 – case-mix adjusted (age, sex, race and ethnicity); and (3) Model 3 – Model 2 + diabetes and dialysis vintage. Correlation analyses were assessed both unadjusted and adjusted for the covariates listed in model 3. P-values were considered statistically significant at the alpha level of 0.05.

All statistical analyses were performed with SAS, version 9.4 (SAS Institute Inc., Cary, NC) and STATA MP version 13.1 (StataCorp, College Station, TX).

RESULTS

Patient Characteristics

In this cohort of 232 randomly selected MADRAD patients, the median (IQR) was 4.72 (3.84, 6.02) pmol/mL for PEA and 6.86 (5.48, 9.15) pmol/mL for OEA. In these MHD patients, the mean±SD age was 54±14 years; 54% were male, 31% were African-American, and 54% were diabetic. Patients with higher serum OEA concentrations were more likely to be older, female or African American, and were less likely to be Caucasian, Hispanic, or diabetic. Compared to patients with lower serum PEA levels, patients with higher serum PEA levels were more likely to be older, female, African American, or diabetic, and were less likely to be Caucasian or Hispanic (Table 1).

Correlations of Serum OEA and PEA with Clinical and Laboratory Measures

Spearman correlation tests showed that serum OEA and PEA levels have a strong positive correlation with each other (Rho: 0.91, p-value: <0.0001) and this is consistent with their common biogenesis and with previously published studies [27, 28]. Without adjusting for covariates, serum OEA positively correlated with HDL-C, LPA-C, and IL-6 (Unadjusted Rho: 0.35, p-value: <0.0001; Rho: 0.13, p-value: 0.04; and Rho: 0.18, p-value: 0.005; respectively) and negatively correlated with triglycerides and VLDL-C (Unadjusted Rho: −0.13, p-value: 0.04 and Rho: −0.25, p-value: 0.0001). After model 3 adjustments, the correlations between LPA-C and triglycerides were no longer significant. Without

adjustments, serum PEA positively correlated with cholesterol and HDL-C (Unadjusted Rho: 0.20, p-value: 0.002 and Rho: 0.32, p-value: <0.0001), and negatively correlated with VLDL-C (Unadjusted Rho: −0.14, p-value: 0.04). The correlation with VLDL-C was no longer significant after adjusting for the model 3 covariates.

When examining the relationship between additional lab variables with serum OEA, a negative correlation was found with creatinine, nPCR, total body water (TBW-Watson), and body weight without adjustments (Unadjusted Rho: −0.23, p-value: 0.001; Rho: −0.19, p-value: 0.006; Rho: −0.21, p-value: 0.003; and Rho: −0.19, p-value: 0.007). The correlation with nPCR was no longer significant after model 3 adjustments. In the unadjusted model, serum PEA was also found to be negatively correlated with creatinine, nPCR, and TBW-Watson (Unadjusted Rho: −0.18, p-value: 0.01; Rho: −0.17, p-value: 0.01 and Rho: −0.17, P-value: 0.02). However, these correlations were no longer significant after model 3 adjustments.

When examining correlations with body anthropometry, serum OEA negatively correlated with mid-arm circumference and biceps average skinfold, without adjustments (Unadjusted Rho: −0.20, p-value: 0.008 and Rho: −0.17, p-value: 0.03). After adjusting for model 3 covariates, near infra-red body fat percentage also became a significant negative correlator with serum OEA (Adjusted Rho: −0.17, p-value: 0.03). In the unadjusted model, serum PEA negatively correlated with mid-arm circumference and biceps average skinfold (Unadjusted Rho: −0.20, p-value: 0.03 and Rho: −0.20, p-value: 0.01). After model 3 adjustments, body fat and triceps average skin fold also became significant negative correlators (Adjusted Rho: −0.18, p-value: 0.02 and Rho: −0.17, p-value: 0.04).

Variables related to quality of life (QOL) scores were also examined. Of these, the Short Form 36 physical functioning subscale was the only QOL variable found to be negatively correlated with PEA after model 3 adjustments (Adjusted Rho: 0.14, p-value: 0.03) and no QOL variables significantly correlated with OEA in both unadjusted and adjusted models (Table 2).

Associations of OEA and PEA with 12-month All-Cause Mortality

In preliminary pilot analyses, we sought to examine the association of circulating OEA and PEA levels with mortality in this small cohort of hemodialysis patients. Throughout the follow-up period of one year, there were a total of 20 mortality events with an incidence rate (95% confidence interval(CI)) of 9.21(5.17, 13.25). There were a total of 6 deaths with an incidence rate of 5.34(1.06, 9.62) for those with OEA<6.9 pmol/mL and 14 deaths with an incidence rate of 13.35(6.36, 20.35) for those with OEA≥6.9 pmol/mL. There were a total of 5 deaths with an incidence rate of 4.66(0.58, 8.75) for those with PEA<4.7 pmol/mL and 15 deaths with an incidence rate of 13.64(6.74, 20.55) for those with PEA≥4.7 pmol/mL (Table 3).

For serum OEA, cubic splines examining the relationship between continuous serum OEA and mortality showed a slightly higher risk in mortality with increasing serum OEA levels in the unadjusted model. This relationship was slightly attenuated with additional levels of adjustment (Fig. 1). When examining the relationship between continuous PEA and

mortality, restricted cubic splines also showed that those with higher serum PEA also had a higher risk for mortality across all models (Fig. 2). In secondary analyses with PEA as a categorical exposure variable, patients with high PEA (PEA≥4.7 pmol/mL) had a higher 12-month all-cause mortality risk compared to the reference group (PEA<4.7 pmol/mL) across all models (hazard ratios [95% CI]: 2.93 [1.07–8.06], 2.86 [1.00–8.15], and 2.99 [1.04-8.64] pmol/mL, respectively). For OEA this mortality risk relationship trended in the same direction, though did not reach statistical significance in all models (hazard ratios [95% CI]: 2.51 [0.96–6.52], 2.10 [0.79–5.63], and 2.16 [0.81-5.78] pmol/mL, respectively) (Table 3).

DISCUSSION

In the present study, we examined the association of two circulating NAEs, OEA and PEA, with clinical and laboratory indices in a cohort of ESRD patients on MHD. In addition, in preliminary hypothesis-generating analyses, we also evaluated the association of serum OEA and PEA levels with all-cause mortality. Our analyses found a positive correlation between serum OEA and PEA levels and HDL-C levels. These findings are aligned with the prior literature which indicates that both of these ligands are activators of the PPAR-α nuclear receptor and that PPAR-α agonism upregulates expression and abundance of apolipoprotein A-1, the major protein constituent of HDL, thereby leading to increased serum levels of HDL-C [18, 29]. This characteristic of OEA and PEA can also explain the negative correlation between these ligands and VLDL-C, given that PPAR-α activation has been shown to result in increased fatty acid oxidation and reduced serum triglycerides [30, 31]. In fact, fibrates are a pharmacologic class of weak PPAR-α agonists used in the treatment of hypertriglyceridemia.

It is interesting that higher circulating levels of OEA and PEA negatively correlate, albeit weakly, with markers of body anthropometry including body fat, mid-arm circumference and biceps skin fold. Although the underlying mechanisms responsible for these findings remain to be elucidated, it should be noted that we found similar negative trends in correlations between AEA and markers of body anthropometry in our previous analyses (although those did not reach statistical significance) [14]. Given the positive correlation of 2-AG with serum triglycerides and markers of body anthropometry, one can speculate that these opposite findings may be related (i.e. increased OEA/PEA levels activate PPAR-α, increased fatty acid oxidation resulting in increased energy metabolism and reduced body mass). In fact, both animal and human studies have found that OEA and PEA play a critical role in satiety and increased levels can be associated with reduced energy intake [32–35]. In addition, recent studies have shown an association between OEA and decreased visceral fat [36].

Another intriguing finding of this study is the weak but positive correlation between circulating OEA and PEA levels and serum pro-inflammatory cytokine IL-6. As mentioned in the introduction, OEA and especially PEA have been shown to suppress inflammation through PPAR-α activation in an NF-κB dependent manner [22, 37, 38]. The underlying mechanism responsible for this positive association requires further investigation, although a previous study showed a positive association between NAEs and IL-6 in the setting of peripheral inflammation in alcohol binge drinkers [39]. Thus, it can be speculated that the

increased levels of OEA and PEA acts as a compensatory mechanism for the increased inflammatory milieu that is a hallmark of ESRD [40]. However, while these lipid-derived mediators may counteract the effect of inflammation, they also activate fatty acid oxidation resulting in reduced serum triglycerides, increased energy expenditure, and decreased body mass. This can be problematic in MHD patients, for which the literature shows that lower serum triglycerides and body mass index are associated with a higher risk of death [10, 41, 42]. Furthermore, we have also previously shown that increasing serum HDL-C levels are paradoxically associated with higher mortality [43, 44]. Although preliminary and in early stages of evaluation, our findings that higher serum levels of OEA and PEA are associated with greater mortality risk is thus consistent with the downstream effects of PPAR-α activation on energy metabolism in ESRD.

Several limitations to our study merit discussion. First, although adjustments were made for potential confounders, residual confounding may remain given unmeasured variables, such as data on hospitalizations, infections, nutrition, and visceral adipose tissue. In addition, given the relatively small sample size, this study may not be adequately powered to detect all significant associations between OEA, PEA, and the measured variables, especially given the few mortality events for risk analysis. Despite this, it should be noted that to our knowledge, this study represents the largest investigation to date on serum levels of these mediators in ESRD patients. Although all serum samples were collected during the day, we cannot rule out an effect from circadian rhythm and timing of blood collection (morning compared to afternoon) in our findings. However, we did reduce the risk of potential variability in the extraction and analysis process given that all serum samples were collected during the day, extracted by one person using the same exact protocol and analyzed on one mass spectrometry device at the same time. Another limitation is the extent to which serum levels of OEA and PEA reflect concentrations in target tissues such as the brain, liver, intestines, and adipose tissue is uncertain [10, 45–48]. Future studies are needed to illuminate the spatial and temporal sources of these mediators as well as their potential diurnal variation [49], all of which may impact their circulating concentrations. Finally, it should be noted that the observational nature of our research precludes making any conclusion about causality. These novel observations are clearly hypothesis-generating; however, future mechanistic studies are needed to further evaluate these findings and to determine whether a causal link exists between serum levels of OEA and PEA and lipid metabolism, inflammation, and mortality in patients on MHD.

In conclusion, we found that serum levels of OEA and PEA, two lipid-derived mediators with modulatory effects on satiety, inflammation and lipid homeostasis, are significantly correlated with variables related to lipid metabolism and body mass. Furthermore, increased serum levels of OEA and PEA are associated with mortality risk in patients on MHD. Further research is needed to confirm our findings and elucidate the potential role of OEA and PEA signaling in relation to energy metabolism and cachexia in patients on MHD.

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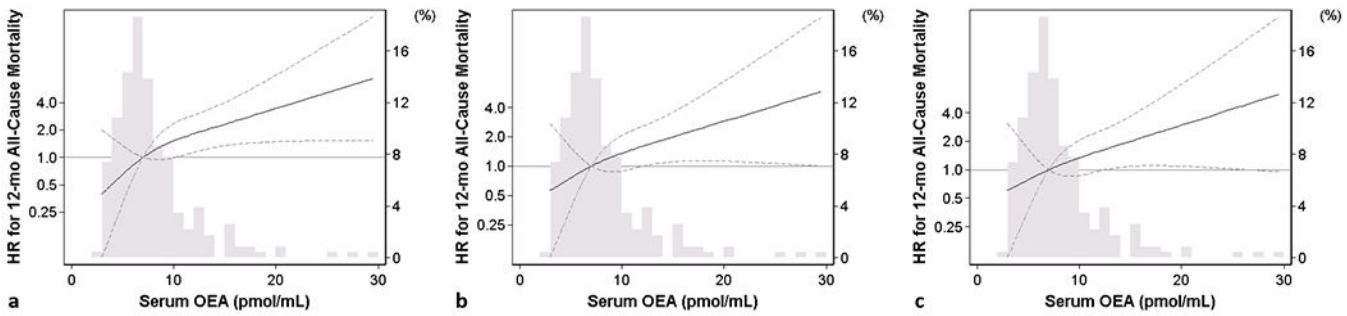

Fig. 1.

Restricted cubic splines of the association between serum OEA and 12-month all-cause mortality among 232 maintenance hemodialysis patients. Splines were adjusted for covariates in models 1-3 as follows: (i) model 1 (unadjusted); (ii) model 2 (age, gender, race and ethnicity); and (iii) model 3 (model 2 + diabetes and dialysis vintage). Solid and dotted lines represent hazard ratios and 95% confidence intervals, respectively. Abbreviations: HR, hazard ratio; mo, month.

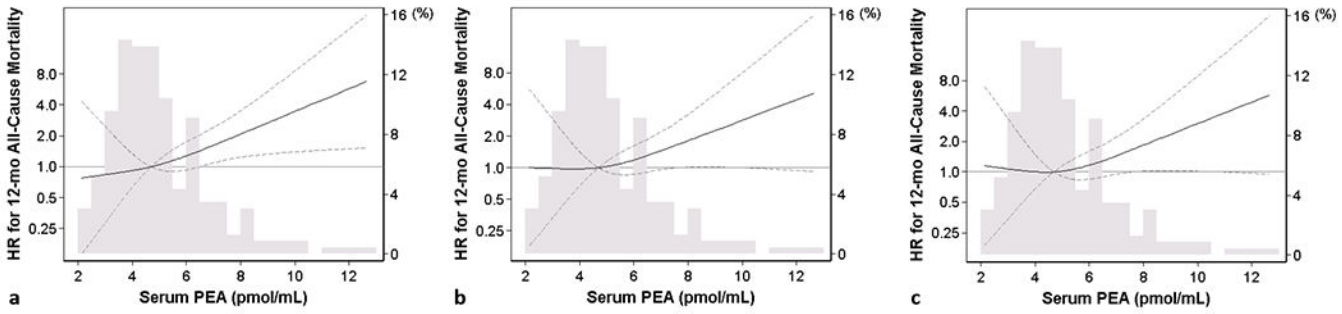

Fig. 2.

Restricted cubic splines of the association between serum PEA and 12-month all-cause mortality among 232 maintenance hemodialysis patients. Splines were adjusted for covariates in models 1-3 as follows: (i) model 1 (unadjusted); (ii) model 2 (age, gender, race and ethnicity); and (iii) model 3 (model 2 + diabetes and dialysis vintage). Solid and dotted lines represent hazard ratios and 95% confidence intervals, respectively. Abbreviations: HR, hazard ratio; mo, month.

Table 1.

Demographic variables and comorbid diabetes categorized by OEA and PEA groups.

		OEA, pmol/mL			PEA, pmol/mL		
Variables	**Total N=232**	**OEA<6.9 N=118**	**OEA≥6.9 N=114**	***p* value**	**PEA<4.9 N=113**	**PEA≥4.9 N=119**	***p* value**
Age	54±14	52±15	56±13	0.05	52±15	56±13	0.03
Male	125(54)	71(60)	54(47)	0.05	68(60)	57(48)	0.06
Race							
Caucasian	149(64)	82(69)	67(59)	0.09	80(71)	69(58)	0.04
African	71(31)	29(25)	42(37)	0.04	26(23)	45(38)	0.01
American							
Hispanic	129(56)	75(66)	54(47)	0.01	72(64)	57(48)	0.02
Asian	12(5)	7(6)	5(4)	0.60	7(6)	5(4)	0.49
Diabetes	114(49)	67(57)	59(52)	0.44	64(57)	62(52)	0.49
OEA, pmol/mL	6.86(5.48,9.15)	5.49(4.46,6.32)	9.29(7.84,12.03)	<.0001	5.48(4.39,6.39)	9.03(7.37,12.03)	<.0001
PEA, pmol/mL	4.72(3.84,6.02)	3.88(3.35,4.32)	6.02(5.04,7.24)	<.0001	3.77(3.33,4.09)	6.02(5.11,7.12)	<.0001
IL-6, pg/mL	2.29(1.16,4.36)	1.87(0.97,4.17)	2.45(1.42,4.87)	0.05	2.03(0.98,4.19)	2.42(1.29,4.41)	0.21
Vintage (%)							
<1 year	5(2)	3(3)	2(2)	0.52	2(2)	3(3)	0.80
1-<3 years	45(19)	25(21)	20(18)	0.48	19(17)	26(22)	0.33
3-<6 years	92(40)	47(40)	45(39)	0.96	47(42)	45(38)	0.56
≥6 years	90(39)	43(36)	47(41)	0.45	45(40)	45(40)	0.75

*Variables are described using Mean±Standard Deviation(SD), Median (Interquartile Range(IQR)), and proportions. Statistical tests for association used were Chi-squared, T-Tests, and Mann-Whitney U Tests as appropriate based on variable type and distribution.

Table 2.

Unadjusted and adjusted Spearman's rank correlation coefficients (Rho) between serum OEA and PEA and data on laboratory, clinical, quality of life and depression tests in 232 maintenance hemodialysis patients.

Variable	OEA, pmol/mL				PEA, pmol/mL			
	unadjusted		adjusted*		unadjusted		adjusted*	
	rho	*p* value	rho	*p* value	rho	*p* value	rho	*p* value
Lipids								
Cholesterol, mg/dL	0.09	0.17	0.06	0.35	0.20	**0.002**	0.17	**0.01**
HDL-C, mg/dL	0.35	**<.0001**	0.29	**<.0001**	0.32	**<.0001**	0.26	**<.0001**
LDL-C, mg/dL	0.01	0.88	0.007	0.91	0.09	0.15	0.09	0.19
Triglycerides, mg/dL	−0.13	**0.04**	−0.11	0.11	−0.05	0.49	−0.01	0.84
LPA-C, mg/dL	0.13	**0.04**	0.05	0.42	0.12	0.08	0.03	0.68
NHDL, mg/dL	−0.10	0.13	−0.10	0.15	0.03	0.67	0.03	0.67
VLDL-C, mg/dL	−0.25	**0.0001**	−0.22	**0.001**	−0.14	**0.04**	−0.10	0.15
Body mass index, kg/m2	−0.12	0.07	−0.13	0.06	−0.07	0.29	−0.08	0.26
IL-6, pg/mL	0.18	**0.005**	0.19	**0.004**	0.12	0.07	0.12	0.07
Laboratory tests								
PEA, pmol/mL	0.91	**<.0001**	0.91	**<.0001**	1	**<.0001**	1	**<.0001**
Albumin, g/dL	−0.12	0.08	−0.05	0.47	−0.07	0.34	0.02	0.78
Creatinine, mg/dL	−0.23	**0.001**	−0.19	**0.009**	−0.18	**0.01**	−0.13	0.08
Ferritin, ng/mL	0.09	0.18	0.06	0.37	0.07	0.35	0.04	0.58
Hemoglobin, g/dL	−0.04	0.61	0.03	0.64	−0.02	0.77	0.05	0.48
Iron saturation, %	−0.03	0.69	0.04	0.60	−0.01	0.85	0.06	0.43
Lymphocytes, %	−0.05	0.46	−0.04	0.58	−0.05	0.47	−0.04	0.54
nPCR, g/kg/d	−0.19	**0.006**	−0.13	0.07	−0.17	**0.01**	−0.10	0.17
TBW, Watson	−0.21	**0.003**	−0.15	**0.03**	−0.17	**0.02**	0.004	0.95
PTH, pg/mL	−0.04	0.58	−0.04	0.56	−0.003	0.96	−0.09	0.19
TIBC, mg/dL	0.002	0.98	0.04	0.56	−0.02	0.79	0.03	0.72
UIBC, µg/dL	0.006	0.93	−0.008	0.91	−0.009	0.90	−0.02	0.74
White blood cell, x1000 mm3	−0.07	0.33	−0.07	0.33	−0.06	0.37	−0.07	−0.34
Weight, kg	−0.19	**0.007**	−0.16	**0.03**	−0.14	0.05	−0.11	0.13

Variable	OEA, pmol/mL				PEA, pmol/mL			
	unadjusted		adjusted*		unadjusted		adjusted*	
	rho	*p* value	rho	*p* value	rho	*p* value	rho	*p* value
Post-dialysis weight, kg	−0.18	**0.008**	−0.15	**0.04**	−0.13	0.06	−0.10	0.18
Pre-dialysis weight, kg	−0.19	**0.007**	−0.15	**0.03**	−0.13	0.06	−0.10	0.17
Body anthropometry measures								
NIR body fat, %	−0.06	0.43	−0.17	**0.03**	−0.07	0.38	−0.18	**0.02**
Mid-arm muscle circ., cm	−0.11	0.17	−0.14	0.07	−0.04	0.62	−0.08	0.34
Mid-arm circ., cm	−0.20	**0.008**	−0.23	**0.003**	−0.17	**0.03**	−0.20	**0.009**
Average biceps skin fold, mm	−0.17	**0.03**	−0.17	**0.03**	−0.20	**0.01**	−0.20	**0.01**
Average triceps skin fold, mm	−0.10	0.18	−0.13	0.10	−0.14	0.08	−0.17	**0.04**
Quality of life measures								
Back Depression Index Score	−0.04	0.59	−0.05	0.51	−0.04	0.56	−0.04	0.51
BDI Score	0.01	0.89	0.003	0.97	−0.02	0.78	−0.03	0.69
Physical Functioning	0.006	0.93	0.08	0.24	0.07	0.29	0.14	**0.03**
Role limitations due to physical health	0.005	0.94	0.06	0.36	0.03	0.66	0.09	0.19
Role limitations due to emotional problems	0.03	0.64	0.06	0.40	0.04	0.53	0.07	0.33
Energy/fatigue	−0.02	0.78	0.006	0.93	0.03	0.67	0.05	0.47
Emotional well-being	0.07	0.27	0.05	0.50	0.07	0.32	0.04	0.59
Social functioning	0.02	0.77	0.03	0.68	0.04	0.58	0.04	0.53
Pain	−0.07	0.27	−0.04	0.57	−0.09	0.18	−0.06	0.42
General Health	0.05	0.44	0.02	0.77	0.11	0.09	0.08	0.22
Physical Health	−0.01	0.87	0.04	0.58	0.03	0.67	0.08	0.25
Mental Health	0.03	0.64	0.04	0.57	0.06	0.33	0.07	0.29

*Results were adjusted for age, gender, race, ethnicity, diabetes and dialysis vintage.

Abbreviations: circ., circumference; HDL-C, high-density lipoprotein-cholesterol; IL-6, Interleukin-6; LDL-C, low-density lipoprotein-cholesterol; LPA-C, lipoprotein(a)-cholesterol; NHDL, non-high-density lipoprotein; NIR, near-infrared; nPCR, normalized protein catabolic rate; PEA, *N*-palmitoylethanolamide; PTH, parathyroid hormone; TBW, total body water (Watson formula); TIBC, total iron-binding capacity; UIBC, unsaturated iron binding capacity; VLDL-C, very low-density lipoprotein-cholesterol.

Table 3.

Cox proportional hazards models for all-cause mortality stratified by OEA and PEA groups. Reference: OEA<6.9 pmol/mL and PEA<4.7 pmol/mL.

	N	Death	Cohort Year	Incidence Rate(95%CI)	Model1		Model 2		Model 3	
					HR(95%CI)	P-Value	HR(95%CI)	P-Value	HR(95%CI)	P-Value
OEA<6.9	118	6	112.28	5.34(1.07, 9.62)	Reference		Reference		Reference	
OEA≥6.9	114	14	104.85	13.35(6.36, 20.35)	2.51(0.96, 6.52)	0.06	2.10(0.79, 5.63)	0.14	2.16(0.80, 5.78)	0.13
PEA <4.7	113	5	107.19	4.66(0.58, 8.75)	Reference		Reference		Reference	
PEA ≥4.7	119	15	109.94	13.64(6.74, 20.55)	2.93(1.07, 8.06)	**0.04**	2.86(1.01, 8.15)	**0.049**	2.99(1.04, 8.64)	**0.04**

*Levels of adjustment are as follows: (1) Model 1 – Unadjusted; (2) Model 2 – case-mix adjusted (age, sex, race and ethnicity); and (3) Model 3 – Model 2 + diabetes and dialysis vintage.