Impact of Circulating *N*-Acylethanolamine Levels with Clinical and Laboratory End Points in Hemodialysis Patients

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Keywords

ESRD · Maintenance hemodialysis · Mortality · Endocannabinoid system · Oleoylethanolamide · Palmitoylethanolamide

Abstract

Background: Patients with 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 has been shown to play an important role in energy metabolism with potential relevance in ESRD. *N*-acylethanolamines are a class of fatty acid amides which include the major endocannabinoid ligand, anandamide, and the endogenous peroxisome proliferator-activated receptor- 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. Secondly, 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 high-density lipoprotein-cholesterol levels and negatively corre-

lated 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 toward higher mortality risk, and these 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 2.99; 95% confidence interval 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.

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Published by S. Karger AG, Basel

Introduction

The population of individuals with advanced CKD and 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, 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 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 – 2-arachidonoyl-sn-glycerol (2-AG) and arachidonylethanolamide (AEA, anandamide) – primarily exert their effects via the canonical cannabinoid receptors 1 and 2. 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 cannabinoid receptors 1 and 2. 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 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 nucle-

ar factor-kappa B (NF- κ B) [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-infrared total body fat percentage. Additional details about depression (Beck Depression Index II) and quality of life (QOL; Short Form 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 h 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 preexisting comorbidity by a combination of patient self-reported history and International Classification of Diseases-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 systems based in Minneapolis, MN, and from Affymetric Thermo Fisher Scientific were used to measure the concentration of serum interleukin-6 (IL-6) by following the manufacturer's protocol.

Lipid Extraction and FAEs and MAGs Analysis

Lipid extraction and analysis was performed as previously described [25, 26]. In 1.0 mL of methanol solution containing the internal standards, [$^2\text{H}_4$]-OEA and [$^2\text{H}_4$]-PEA (Cayman Chemi-

cal, Ann Arbor, MI, USA), 0.5 mL of serum was placed. 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 ultrahigh purity N₂ stream (99.998% pure) and resuspended in 0.1 mL of methanol:chloroform (9:1), with 1 µL 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 Quadrupole Mass Spectrometer (Waters Corporation, Milford, MA, USA) with electrospray ionization sample delivery. Lipids were separated using an Acquity UPLC BEH C₁₈ column (2.1 × 50 mm i.d., 1.7 µm, Waters) and in-line Acquity guard column (UPLC BEH C₁₈ VanGuard PreColumn; 2.1 × 5 mm i.d.; 1.7 µm, Waters) and eluted by a gradient of water and methanol (containing 0.25% acetic acid and 5 mM ammonium acetate) at a flow rate of 0.4 mL/min and gradient: 80% methanol 0.5 min, 80–100% methanol 0.5–2.5 min, 100% methanol 2.5–3.0 min, 100–80% methanol 3.0–3.1 min, and 80% methanol 3.1–4.5 min. 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 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, ($m/z = 300.3 > 62.1$), [²H₄]-OEA ($m/z = 330.4 > 66.0$), and [²H₄]-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), or proportions, as appropriate based on variable type and distribution. χ^2 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 QOL 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, USA) and STATA MP version 13.1 (StataCorp, College Station, TX, USA).

Results

Patient Characteristics

In this cohort of 232 randomly selected MADRAD patients, the median (interquartile range) 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

Table 1. Demographic variables and comorbid diabetes categorized by OEA and PEA groups

Variable	Total N = 232	OEA		p value	PEA		p value
		OEA <6.9 pmol/mL N = 118	OEA ≥6.9 pmol/mL N = 114		PEA <4.9 pmol/mL N = 113	PEA ≥4.9 pmol/mL N = 119	
Age, years	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 American	71 (31)	29 (25)	42 (37)	0.04	26 (23)	45 (38)	0.01
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)	<0.0001	5.48 (4.39, 6.39)	9.03 (7.37, 12.03)	<0.0001
PEA, pmol/mL	4.72 (3.84, 6.02)	3.88 (3.35, 4.32)	6.02 (5.04, 7.24)	<0.0001	3.77 (3.33, 4.09)	6.02 (5.11, 7.12)	<0.0001
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 to <3 years	45 (19)	25 (21)	20 (18)	0.48	19 (17)	26 (22)	0.33
3 to <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

OEA, oleoylethanolamide; PEA, *N*-palmitoylethanolamide; IL-6, interleukin-6. Variables are described using mean ± standard deviation (SD), median (interquartile range [IQR]), and proportions. Statistical tests for association used were χ^2 , *t* tests, and Mann-Whitney U tests as appropriate based on variable type and distribution.

ed 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 Cr, nPCR, total body water (Watson formula), 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 Cr, nPCR, and total body water (Watson formula; 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-infrared 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 skinfold also became significant negative correlators (adjusted rho: −0.18, *p* value: 0.02 and rho: −0.17, *p* value: 0.04).

Variables related to 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 1 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

Table 2. Unadjusted and adjusted Spearman's rank correlation coefficients (rho) between serum OEA and PEA and data on laboratory, clinical, QOL, and depression tests in 232 MHD patients

Variable	OEA, pmol/mL				PEA, pmol/mL			
	unadjusted		adjusted ^a		unadjusted		adjusted ^a	
	rho	p value	rho	p value	rho	p value	rho	p value
Lipids, mg/dL								
Cholesterol	0.09	0.17	0.06	0.35	0.20	0.002	0.17	0.01
HDL-C	0.35	<0.0001	0.29	<0.0001	0.32	<0.0001	0.26	<0.0001
LDL-C	0.01	0.88	0.007	0.91	0.09	0.15	0.09	0.19
Triglycerides	-0.13	0.04	-0.11	0.11	-0.05	0.49	-0.01	0.84
LPA-C	0.13	0.04	0.05	0.42	0.12	0.08	0.03	0.68
NHDL	-0.10	0.13	-0.10	0.15	0.03	0.67	0.03	0.67
VLDL-C	-0.25	0.0001	-0.22	0.001	-0.14	0.04	-0.10	0.15
BMI, kg/m ²	-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	<0.0001	0.91	<0.0001	1	<0.0001	1	<0.0001
Albumin, g/dL	-0.12	0.08	-0.05	0.47	-0.07	0.34	0.02	0.78
Cr, 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/day	-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, ×1,000/mm ³	-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
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 skinfold, 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
QOL measures								
Beck 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

QOL, quality of life; MHD, maintenance hemodialysis; 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. Values in bold indicate statistical significance. ^a Results were adjusted for age, gender, race, ethnicity, diabetes, and dialysis vintage.

Fig. 1. Restricted cubic splines of the association between serum OEA and 12-month all-cause mortality among 232 MHD 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 HRs and 95% CIs, respectively. OEA, oleoylethanolamide; MHD, maintenance hemodialysis; HR, hazard ratio; CI, confidence interval; mo, month.

Table 3. Cox proportional hazards models for all-cause mortality stratified by OEA and PEA groups

	N	Death	Cohort year	Incidence rate (95% CI)	Model 1		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

Reference: OEA <6.9 pmol/mL and PEA <4.7 pmol/mL. OEA, oleoylethanolamide; PEA, N-palmitoylethanolamide; HR, hazard ratio; CI, confidence interval. 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. Values in bold indicate statistical significance.

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 2 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

Fig. 2. Restricted cubic splines of the association between serum PEA and 12-month all-cause mortality among 232 MHD 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 HRs and 95% CIs, respectively. PEA, *N*-palmitoylethanolamide; MHD, maintenance hemodialysis; HR, hazard ratio; CI, confidence interval; mo, month.

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 skinfold. 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- κ B 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 act 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 BMI 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, 2 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.

Acknowledgements

The project described was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences at the National Institutes of Health through grant UL1 TR001414. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. We are also grateful to the UC Irvine Institute for Clinical and Translational Science for their support. HM is supported by a career development award from the Office of Research and Development of the Department of Veterans Affairs (1 IK CX

001043-01). E.S. is supported by a career development award from the Office of Research and Development of the Department of Veterans Affairs (IK2-CX001266-01). K.K.Z. is supported by NIH (NIDDK) grants K24-DK091419 and R01-DK078106, and also by philanthropic grants from Mr. Harold Simmons, Mr. Louis Chang, Joseph Lee, and AVEO, Inc. C.M.R. is supported by the NIH (NIDDK) grants R01-DK122767 and R03-DK114642. The NVD laboratory is supported by NIH grants R01-DK119498 and L30-DK114978 (NIDDK), and Tobacco-Related Disease Research Program grant T29KT0232. H.M., E.S., and K.K.Z. are employees of the U.S. Department of Veterans Affairs. The opinions expressed in this article are those of the authors and do not represent the views of the U.S. Department of Veterans Affairs or the U.S. government.

Statement of Ethics

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. The patients provided written/signed informed consent.

Conflict of Interest Statement

K.K.Z., H.M., and D.P. 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.

Funding Sources

H.M. has received funding from the NIH, VA ORD, Amgen, and Novartis. C.M.R. has received honoraria and/or support from AstraZeneca, Fresenius, Ostuka, Reata, and Roche. K.K.Z. has received honoraria and/or support from Abbott, Abbvie, Alexion, American Society of Nephrology, Amgen, AstraZeneca, AVEO, Chugai, DaVita, Fresenius, Genetech, Haymarket Media, Hospira, Kabi, Keryx, National Institutes of Health, National Kidney Foundation, Relypsa, Resverlogix, Sanofi, Shire, Vifor, and ZS-Pharma.

Author Contributions

Research idea and study design: H.M., D.P., and K.K.Z.; data acquisition: H.M., E.S., and C.M.R.; data analysis/interpretation: A.P., E.S., D.A.A., and H.M.; statistical analysis: A.P., C.W., E.S., and H.M.; supervision or mentorship: D.P., K.K.Z., N.D.V., and N.V.D. Each author contributed important intellectual content during manuscript drafting or revision, accepts personal accountability for the author's own contributions, and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

References

- McCullough KP, Morgenstern H, Saran R, Herman WH, Robinson BM. Projecting ESRD incidence and prevalence in the United States through 2030. *J Am Soc Nephrol*. 2019; 30(1):127–35.
- Kovesdy CP, Kalantar-Zadeh K. Enter the dragon: a Chinese epidemic of chronic kidney disease? *Lancet*. 2012;379(9818):783–5.
- Prospective Studies C, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet*. 2009; 373(9669):1083–96.
- Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA*. 2013;309(1):71–82.
- Yamauchi Y, Hasegawa W, Yasunaga H, Sunohara M, Jo T, Takami K, et al. Paradoxical association between body mass index and in-hospital mortality in elderly patients with chronic obstructive pulmonary disease in Japan. *Int J Chron Obstruct Pulmon Dis*. 2014; 9:1337–46.
- van der Sande MA, Schim van der Loeff MF, Aveika AA, Sabally S, Togun T, Sarge-Njie R, et al. Body mass index at time of HIV diagnosis: a strong and independent predictor of survival. *J Acquir Immune Defic Syndr*. 2004; 37(2):1288–94.
- Kalantar-Zadeh K, Block G, Horwich T, Fonarow GC. Reverse epidemiology of conventional cardiovascular risk factors in patients with chronic heart failure. *J Am Coll Cardiol*. 2004;43(8):1439–44.
- Park J, Ahmadi SF, Streja E, Molnar MZ, Flegal KM, Gillen D, et al. Obesity paradox in end-stage kidney disease patients. *Prog Cardiovasc Dis*. 2014;56(4):415–25.
- Naderi N, Kleine CE, Park C, Hsiung JT, Soohoo M, Tantisattamo E, et al. Obesity paradox in advanced kidney disease: from bedside to the bench. *Prog Cardiovasc Dis*. 2018 Jul–Aug;61(2):168–81.
- Hillard CJ. Circulating endocannabinoids: from whence do they come and where are they going? *Neuropsychopharmacol*. 2018; 43(1):155–72.
- DiPatrizio NV, Piomelli D. The thrifty lipids: endocannabinoids and the neural control of energy conservation. *Trends Neurosci*. 2012; 35(7):403–11.
- Kim J, Li Y, Watkins BA. Endocannabinoid signaling and energy metabolism: a target for dietary intervention. *Nutrition*. 2011;27(6): 624–32.
- Moradi H, Park C, Igarashi M, Streja E, Argueta DA, Soohoo M, et al. Serum endocannabinoid levels in patients with end-stage renal disease. *J Endocr Soc*. 2019;3(10):1869–80.
- Moradi H, Park C, Streja E, Argueta DA, DiPatrizio NV, You AS, et al. Circulating endocannabinoids and mortality in hemodialysis patients. *Am J Nephrol*. 2020;51(2):86–95.
- Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodríguez De Fonseca F, et al. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR- α . *Nature*. 2003;425(6953):90–3.
- LoVerme J, La Rana G, Russo R, Calignano A, Piomelli D. The search for the palmitoylethanolamide receptor. *Life Sci*. 2005;77(14): 1685–98.
- Tsuboi K, Uyama T, Okamoto Y, Ueda N. Endocannabinoids and related N-acylethanolamines: biological activities and metabolism. *Inflamm Regen*. 2018;38:28.
- Karwad MA, Macpherson T, Wang B, Theophilidou E, Sarmad S, Barrett DA, et al. Oleoylethanolamine and palmitoylethanolamine modulate intestinal permeability in vitro via TRPV1 and PPAR α . *FASEB J*. 2017; 31(2):469–81.
- Pesce M, D'Alessandro A, Borrelli O, Gigli S, Seguela L, Cuomo R, et al. Endocannabinoid-related compounds in gastrointestinal diseases. *J Cell Mol Med*. 2018;22(2):706–15.
- Ho WS, Barrett DA, Randall MD. 'Entourage' effects of N-palmitoylethanolamide and N-oleoylethanolamide on vasorelaxation to anandamide occur through TRPV1 receptors. *Br J Pharmacol*. 2008;155(6):837–46.
- Varga T, Czimmerer Z, Nagy L. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim Biophys Acta*. 2011;1812(8):1007–22.
- Huang D, Zhao Q, Liu H, Guo Y, Xu H. PPAR- α agonist WY-14643 inhibits LPS-induced inflammation in synovial fibroblasts via NF- κ B pathway. *J Mol Neurosci*. 2016; 59(4):544–53.
- Kytikova OY, Perelman JM, Novgorodtseva TP, Denisenko YK, Kolosov VP, Antonyuk MV, et al. Peroxisome proliferator-activated receptors as a therapeutic target in asthma. *PPAR Res*. 2020;2020:8906968.
- Rhee CM, Nguyen DV, Moradi H, Brunelli SM, Dukkkipati R, Jing J, et al. Association of adiponectin with body composition and mortality in hemodialysis patients. *Am J Kidney Dis*. 2015;66(2):313–21.
- Batugedara HM, Argueta D, Jang JC, Lu D, Macchietto M, Kaur J, et al. Host- and helminth-derived endocannabinoids that have effects on host immunity are generated during infection. *Infect Immun*. 2018;86(11): e00441–18.
- Angelini R, Argueta DA, Piomelli D, DiPatrizio NV. Identification of a widespread palmitoylethanolamide contamination in standard laboratory glassware. *Cannabis Cannabinoid Res*. 2017;2(1):123–32.
- Bruun S, Gouveia-Figueira S, Domellöf M, Husby S, Neergaard Jacobsen L, Michaelsen KF, et al. Satiety factors oleoylethanolamide, stearoylethanolamide, and palmitoylethanolamide in mother's milk are strongly associated with infant weight at four months of age: data from the odense child cohort. *Nutrients*. 2018;10(11):1747.
- Stensson N, Ghafouri B, Gerdle B, Ghafouri N. Alterations of anti-inflammatory lipids in plasma from women with chronic widespread pain: a case control study. *Lipids Health Dis*. 2017;16(1):112.
- Khera AV, Millar JS, Ruotolo G, Wang MD, Rader DJ. Potent peroxisome proliferator-activated receptor- α agonist treatment increases cholesterol efflux capacity in humans with the metabolic syndrome. *Eur Heart J*. 2015; 36(43):3020–2.
- Finck BN, Chinetti G, Staels B. PPARs/RXRs in cardiovascular physiology and disease. *PPAR Res*. 2008;2008:173780.
- Guzmán M, Lo Verme J, Fu J, Oveisi F, Blázquez C, Piomelli D. Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR- α). *J Biol Chem*. 2004 Jul 2;279(27):27849–54.
- Lucanic M, Held JM, Vantipalli MC, Klang IM, Graham JB, Gibson BW, et al. N-acylethanolamine signalling mediates the effect of diet on lifespan in *Caenorhabditis elegans*. *Nature*. 2011;473(7346):226–9.
- Jones PJ, Lin L, Gillingham LG, Yang H, Omar JM. Modulation of plasma N-acylethanolamine levels and physiological parameters by dietary fatty acid composition in humans. *J Lipid Res*. 2014;55(12):2655–64.
- Hansen HS. Role of anorectic N-acylethanolamines in intestinal physiology and satiety control with respect to dietary fat. *Pharmacol Res*. 2014;86:18–25.
- Fu J, Oveisi F, Gaetani S, Lin E, Piomelli D. Oleoylethanolamide, an endogenous PPAR- α agonist, lowers body weight and hyperlipidemia in obese rats. *Neuropharmacology*. 2005 Jun;48(8):1147–53.
- Bowen KJ, Kris-Etherton PM, Shearer GC, West SG, Reddivari L, Jones PJH. Oleic acid-derived oleoylethanolamide: a nutritional science perspective. *Prog Lipid Res*. 2017;67:1–15.
- Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A, et al. The nuclear receptor peroxisome proliferator-activated receptor- α mediates the anti-inflammatory actions of palmitoylethanolamide. *Mol Pharmacol*. 2005;67(1):15–9.
- Payahoo L, Khajebishak Y, Asghari Jafarabadi M, Ostadrahimi A. Oleoylethanolamide supplementation reduces inflammation and oxidative stress in obese people: a clinical trial. *Adv Pharm Bull*. 2018;8(3):479–87.

- 39 Anton M, Rodriguez-Gonzalez A, Rodriguez-Rojo IC, Pastor A, Correias A, Serrano A, et al. Increased plasma oleoylethanolamide and palmitoleoylethanolamide levels correlate with inflammatory changes in alcohol binge drinkers: the case of HMGB1 in women. *Addict Biol.* 2018;23(6):1242–50.
- 40 Cobo G, Lindholm B, Stenvinkel P. Chronic inflammation in end-stage renal disease and dialysis. *Nephrol Dial Transpl.* 2018;33:35–40.
- 41 Calabia J, Arcos E, Carrero JJ, Comas J, Vallés M. Does the obesity survival paradox of dialysis patients differ with age? *Blood Purif.* 2015; 39(1–3):193–9.
- 42 Polinder-Bos HA, van Diepen M, Dekker FW, Hoogeveen EK, Franssen CFM, Gansevoort RT, et al. Publisher correction: lower body mass index and mortality in older adults starting dialysis. *Sci Rep.* 2018;8(1):14231.
- 43 Moradi H, Streja E, Kashyap ML, Vaziri ND, Fonarow GC, Kalantar-Zadeh K. Elevated high-density lipoprotein cholesterol and cardiovascular mortality in maintenance hemodialysis patients. *Nephrol Dial Transplant.* 2014;29(8):1554–62.
- 44 Moradi H, Streja E, Kalantar-Zadeh K. Serum high density lipoprotein cholesterol level and risk of death: let's avoid the extremes. *J Thorac Dis.* 2017;9(12):4849–52.
- 45 Leung D, Saghatelian A, Simon GM, Cravatt BF. Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry.* 2006;45(15):4720–6.
- 46 Joosten MM, Balvers MG, Verhoeckx KC, Hendriks HF, Witkamp RF. Plasma anandamide and other N-acyl ethanolamines are correlated with their corresponding free fatty acid levels under both fasting and non-fasting conditions in women. *Nutr Metab.* 2010;7:49.
- 47 Artmann A, Petersen G, Hellgren LI, Boberg J, Skonberg C, Nellemann C, et al. Influence of dietary fatty acids on endocannabinoid and N-acyl ethanolamine levels in rat brain, liver and small intestine. *Biochim Biophys Acta.* 2008;1781(4):200–12.
- 48 Inoue M, Tsuboi K, Okamoto Y, Hidaka M, Uyama T, Tsutsumi T, et al. Peripheral tissue levels and molecular species compositions of N-acyl-phosphatidylethanolamine and its metabolites in mice lacking N-acyl-phosphatidylethanolamine-specific phospholipase D. *J Biochem.* 2017;162(6):449–58.
- 49 Hanlon EC. Impact of circadian rhythmicity and sleep restriction on circulating endocannabinoid (eCB) N-arachidonylethanolamine (anandamide). *Psychoneuroendocrinology.* 2020;111:104471.