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Authors

Moradi, Hamid

Park, Christina

Streja, Elani

et al.

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Circulating Endocannabinoids and Mortality in Hemodialysis Patients

Hamid Moradi,[a,c,*] Christina Park,[a] Elani Streja,[a,c] Donovan A. Argueta,[d] Nicholas V. DiPatrizio,[d] Amy S. You,[a] Connie M. Rhee,[a] Nosratola D. Vaziri,[a] Kamyar Kalantar-Zadeh,[a,c] and Daniele Piomelli[b]

[a]Division of Nephrology, Hypertension and Kidney Transplantation, University of California Irvine School of Medicine, Irvine, California, USA
[b]Anatomy and Neurobiology, University of California Irvine School of Medicine, Irvine, California, USA
[c]Tibor Rubin VA Medical Center, Nephrology Section, Long Beach, California, USA
[d]Division of Biomedical Sciences, University of California Riverside School of Medicine, Riverside, California, USA
*Hamid Moradi, MD, Veterans Affairs Long Beach Healthcare System, 5901 East 7th Street, Long Beach, CA 90822 (USA), E-Mail hamid.moradi@va.gov

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Abstract

Background

Mortality in patients with end-stage renal disease (ESRD) on maintenance hemodialysis (MHD) remains exceptionally high. While traditional risk factors such as obesity are paradoxically associated with better survival, nontraditional risk factors including cachexia increase the likelihood of poor outcomes. There is accumulating evidence that the endocannabinoid (ECB) system plays a major role in energy preservation and storage, factors which can prevent the deleterious effects of cachexia. Hence, in this study, we evaluated the association of circulating ECB levels with mortality in MHD patients.

Methods

Serum concentrations of anandamide (AEA) and 2-arachidonoyl-*sn*−glycerol (2-AG), major ECB ligands, were measured in MHD patients. Their correlation with various clinical/laboratory indices and association with 12-month all-cause mortality were examined.

Results

Serum 2-AG levels positively correlated with body mass index, serum triglycerides and body anthropometric measures. Meanwhile, serum AEA levels correlated positively with serum interleukin-6, and negatively with serum very low-density lipoprotein levels. While increased serum 2-AG levels were associated with reduced risk of all-cause mortality (hazard ratio [HR] 0.52, 95% CI 0.28–0.98), there was no clear association between serum AEA levels and mortality (HR 0.91, 95% CI 0.48–1.72).

Conclusions

In MHD patients, the circulating levels of ECB ligand, 2-AG, may play an important role in determining body mass and risk of mortality. These observations were unique to 2-AG as similar findings were not obtained with serum AEA. Future studies need to investigate the mechanisms responsible for these associations and examine the modulation of the ECB system as a potential target for therapy in ESRD.

Keywords: End stage renal disease, Hemodialysis, Mortality, Endocannabinoid, Endocannabinoid system, 2-arachidonoylglycerol, Anandamide

Introduction

The prevalence of end-stage renal disease (ESRD) continues to increase in the United States with recent projections estimating that by 2030 there will >970,000 patients with ESRD requiring renal replacement therapy including maintenance hemodialysis (MHD) [1, 2, 3, 4]. Patients on MHD experience a significantly increased risk of death and treatment of traditional risk factors such as hyperlipidemia is not necessarily associated with improved outcomes in this patient population [5, 6]. In fact, the presence of certain traditional risk factors including obesity and hypertriglyceridemia can be paradoxically associated with better survival [7, 8, 9, 10], whereas nontraditional risk factors such as inefficient energy metabolism as manifested by protein energy wasting (PEW) and cachexia increase the risk for worse outcomes [8, 11, 12]. It is well known that ESRD is associated with a catabolic state marked by increased basal energy expenditure, which leads to the wasting of adipose tissue and skeletal muscle (i.e., cachexia) [11, 12, 13] and poor outcomes including a significantly higher risk of death [11, 14, 15].

In this context, one promising area that has not been fully explored is the role of the endocannabinoid (ECB) system in ESRD-associated cachexia and mortality. It is well-established that the ECB system plays an important role in energy metabolism [16, 17]. The latter effects are mediated via central and peripheral mechanisms relying on activation of specific G protein-coupled receptors, cannabinoid-1 (CB_1R) and cannabinoid-2, by endogenous lipid-derived ligands (ECBs). Anandamide (also known as N-arachidonoylethanolamine or AEA) and 2-arachidonoyl-*sn*−glycerol (2-AG) are the most comprehensively studied ECB ligands and significant levels of these lipid-derived mediators have been found in the circulation as well as most organs including the brain, liver, and adipocytes [17, 18, 19, 20]. The activation of the ECB system (mainly via CB_1R activation) leads to an increased intake of energy-rich foods, decreased energy expenditure by inhibition of brown adipose tissue activity, and stimulation of energy storage via lipogenesis and white adipose tissue formation [20, 21]. Overactivity of the ECB system has been demonstrated to

play a causal role in pathogenesis of obesity, hypertriglyceridemia and metabolic syndrome and there is accumulating evidence that obesity and metabolic syndrome are associated with significant elevations of circulating ECBs [22, 23, 24]. Conversely, pharmacological antagonists of CB_1R have been shown to decrease body weight and improve metabolic profile in obese animals and humans [20, 25, 26, 27]. While the role of the ECB system in the pathogenesis of obesity and metabolic syndrome has been well-described, its potential alterations and utility as a therapeutic target in the context of cachexia and wasting has been less studied. This is especially significant in conditions where PEW and cachexia can make an important contribution to the risk of morbidity and mortality such as ESRD. We recently found significant circulating levels of 2-AG in serum of patients on hemodialysis [28]. Serum levels of 2-AG positively correlated with serum triglycerides and markers of body mass [28]. However, given the small sample size of studies which have evaluated circulating ECBs, the association of serum levels of these mediators with clinical endpoints including mortality is lacking.

In light of our recent findings and given the association of ESRD with abnormal energy metabolism and PEW and the role of ECB system in energy homeostasis, we sought to further evaluate the association of circulating ECB levels with laboratory and clinical outcomes including all-cause mortality in a large sample of patients on hemodialysis.

Methods

Study Population

We identified a random subcohort of MHD adult patients actively participating in the ongoing, prospective Malnutrition, Diet and Racial Disparities in Chronic Kidney Disease (MADRAD) study (ClinicalTrials.gov, NCT01415570) between 2012 and 2015. In the MADRAD study, differences in dietary factors and nutritional status are examined across racial and ethnic groups of MHD patients receiving treatment in outpatient dialysis facilities in the Southern California region [29].

There were 523 unique patients who were enrolled in MADRAD and had a blood measurement taken between October 2013 and 2015. Among these patients, we pulled 450 random samples for analysis. After removing patient samples with measurement errors due to the extraction process or sample loss, 400 and 436 MHD patients had data available to analyze serum 2-AG and AEA respectively.

This study was approved by the Institutional Review Committees of the Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA, USA, and the University of California, Irvine Medical Center, Orange, CA, USA. Patients were included in the study if they provided written/signed informed consent.

Demographic, Clinical and Laboratory Measurements

MADRAD study coordinators collected baseline data on demographic and clinical measures. Data on body mass index (BMI), using post-dialysis weight, were primarily obtained from the electronic records of a large dialysis organization, and supplemented with available BMI levels collected by MADRAD study coordinators. Additional details about body anthropometry ascertainment and depression and quality of life measures have been previously reported [29, 30].

Using large dialysis organization electronic records, we extracted routine laboratory measurements obtained closest to the blood draw dates for baseline 2-AG or AEA in all analyses. For laboratory analyses, predialysis non-fasting blood samples were drawn using standardized techniques and measured using automated and standardized methods at a central laboratory in Deland, FL, typically within 24 h. We calculated dialysis vintage for MHD patients as the time between the dates of the patient's first dialysis treatment and serum AG or AEA measurement. The presence of diabetes as a preexisting comorbidity was determined at the time of study entry by a combination of patient self-reported history and International Classification of Diseases-9 codes by MADRAD study coordinators.

Serum Samples and Analyses

Serum concentrations of interleukin (IL)-6 were measured using ELISA assay kits (R&D systems, Minneapolis, MN, USA, and Affymetrix ThermoFisher Scientific) per manufacturer's protocol. For ECB analyses, non-fasting serum was obtained from patients before weekly dialysis sessions, coinciding with routine blood tests performed at the outpatient dialysis facilities, and frozen at −80°C until analysis. Analysis of 2-AG and AEA was performed using ultra performance liquid chromatography − tandem mass spectrometry (UPLC/MS/MS) as previously described [30]. For the assay, the established lower limit of quantitation (signal-to-noise ratio of N10) of analytes using our optimized UPLC/MS/MS methods were as follows: 2-AG, 0.5 pmol; AEA, 0.008 pmol [31]. For further details, please see the online supplementary Material (for all online suppl. material, see www.karger.com/doi/10.1159/000505444.

Exposure and Outcome Ascertainment

The primary exposures of interest were continuous serum 2-AG and AEA. We also created categorical exposure variables of serum 2-AG and AEA by dichotomizing serum 2-AG and AEA levels at their median values (<32.5 and ≥32.5 pmol/mL; <1.06 and ≥1.06 pmol/mL respectively).

The primary outcome was 12-month all-cause mortality. Patients were followed from date of measured serum 2-AG or serum AEA to death, transplantation, loss-to-follow-up, end-of-study period (June 30, 2018 for both) or 12-month follow-up, whichever occurred first. Information on mortality and censored events was collected every 6 months by MADRAD study coordinators and reviewed by MADRAD study nephrologists (C.M.R. and K.K.-Z.).

Statistical Analysis

Baseline patient characteristics are presented according to 2-AG and AEA categories. We used mean ± SD or median (interquartile range) for continuous variables and proportions for categorical variables, where appropriate. Outliers less than the 0.5th or greater than the 99.5th percentiles of laboratory data were removed and replaced with their respective lower or upper threshold values. Most often 2 or less lab values were updated, with the exception of biceps skin fold and BMI, which had 4 and 3 values replaced respectively. Absolute standardized differences were used for comparison of baseline characteristics between 2-AG and AEA categories, separately. Imbalance of patient characteristics across exposure categories was defined as an absolute value of the standardized difference >0.20. We tested the normal distributions of serum 2-AG and AEA using Shapiro-Wilk tests.

We used Spearman's rank correlation coefficients (Rho) to evaluate the relationship between clinical, laboratory, quality of life and depression measures with non-normally distributed serum 2-AG and AEA levels.

Using Cox proportional hazards models with restricted cubic spline functions, we examined the associations of continuous serum 2-AG and AEA levels with 12-month all-cause mortality using 4 adjustment models: (1) Model 1 – Unadjusted; (2) Model 2 – case-mix adjusted (age, sex, race and ethnicity); (3) Model 3 – Model 2 + diabetes and dialysis vintage; and (4) Model 4 – Model 3 + IL-6. Correlation analyses were performed with model 3 adjustment. As secondary analyses, associations of serum 2-AG and AEA as categorical variables with 12-month all-cause mortality were also assessed across the 4 adjustment models.

Missing data for IL-6 in the 2-AG and AEA cohorts (20 and 25% of data, respectively) were imputed by the mean. Two-sided p values <0.05 were considered statistically significant. 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

The final study populations included 400 patients with serum 2-AG measurements and 436 patients with serum AEA measurements. Table 1 shows the baseline characteristics of 400 patients in the 2-AG cohort and 436 patients in the AEA cohort respectively. Among 400 MHD patients, the mean ± SD age was 55 ± 14 years; 45% were female, 29% were African-American and 54% were diabetic. Patients with higher serum 2-AG concentrations were more likely to be African-American, have higher levels of BMI, total iron-binding capacity, triglycerides and cholesterol, but lower high-density lipoprotein-cholesterol (HDL-C; absolute standardized difference >0.2). Of the 436 patients with serum AEA levels, the mean ± SD age was 55 ± 14 years; 44% were female, 30% were African-American and 54% were diabetic. Compared to patients with lower serum AEA levels, patients with higher serum AEA levels were more likely to be older and have higher IL-6 concentrations, but lower levels of very low-density lipoprotein-cholesterol (VLDL-C) and creatinine.

Correlations of Serum 2-AG and AEA with Clinical and Laboratory Measures

We observed correlations between serum 2-AG and all lipids after adjustment for case-mix plus diabetes and dialysis vintage variables. Spearman correlation coefficients for lipoprotein(a)-cholesterol, low-density lipoprotein-cholesterol and total cholesterol were weak (Spearman's rho coefficients, −0.10, 0.12, and 0.15 respectively), whereas HDL-C, VLDL-C and triglycerides modestly correlated with serum 2-AG concentrations (Spearman's rho coefficients, −0.34, 0.41, and 0.47, respectively; Table 2). Body anthropometric measures including BMI, average triceps skin fold and average biceps skin fold correlated positively with serum 2-AG levels (Spearman's rho coefficients, 0.18, 0.22, and 0.23, respectively). Results of Spearman correlation coefficients of 2-AG with additional laboratory tests, quality of life and depression data are presented in online supplementary Table S1. Serum 2-AG levels significantly correlated with unsaturated iron binding capacity, total body water, and patient weight.

Spearman correlation coefficients between serum AEA and creatinine, triglycerides and VLDL-C were around −0.1 (Spearman's rho coefficients, −0.12, −0.13, and −0.18 respectively; Table 2) after adjustment for case-mix plus diabetes and dialysis vintage covariates, whereas, IL-6 positively correlated with serum AEA concentrations (Spearman's rho coefficient, 0.18). Body anthropometric data did not correlate with serum AEA levels. In online supplementary Table S2, additional Spearman correlation coefficients of AEA and other relevant laboratory tests, quality of life and depression data are reported.

Associations of Serum 2-AG and AEA with 12-Month All-Cause Mortality Risk

Among 400 patients in whom serum 2-AG levels were measured, 43 death events occurred during 1 year of follow-up with an incidence rate (95% CI) of 11.7 (8.2–15.2). Restricted cubic splines showed that patients with serum 2-AG levels >32.5 pmol/mL (reference) had lower 12-month mortality risk across adjustment models 2–4 (Fig. 1). In secondary analyses with serum 2-AG as a categorical exposure variable, patients with high serum 2-AG (≥32.5 pmol/mL) had lower 12-month all-cause mortality risk compared to the reference group of low serum 2-AG (<32.5 pmol/mL) in models 3 and 4 (hazard ratios [95% CI] 0.52 [0.28–0.99] and 0.52 [0.28–0.98] pmol/mL respectively; online suppl. Fig. S1, Table S3).

In 436 MHD patients with serum AEA levels, 47 death events occurred during 1 year of follow-up with an incidence rate of 11.7 (8.4–15.1). Results for serum AEA using restricted cubic spline models suggested that higher serum AEA levels trended towards higher 12-month mortality risk across all models (Fig. 2). However, we found no associations between serum AEA as a categorical variable and 12-month mortality risk (online suppl. Fig. S2, Table S4).

Discussion

It has been shown that non-traditional risk factors such as impaired energy metabolism, PEW and cachexia make a substantial contribution to the disproportionately elevated risk of death in patients with ESRD being treated with MHD. In this regard, activation of the ECB system results in in-

creased energy intake, generation and storage and overactivity of this system (as indicated by elevated circulating ECB levels) has been shown to be associated with obesity and hyperlipidemia [20]. However, the association of circulating ECB levels with mortality has not been evaluated in patients with ESRD. In this study, we confirmed our previous findings in a larger set of MHD patients and found that serum levels of a major ECB ligand, 2-AG, positively correlate with higher serum triglyceride levels, increased BMI and higher body mass [30]. These findings are consistent with available literature on the role of circulating ECBs in pathogenesis of obesity and metabolic syndrome. However, we also found that higher serum 2-AG levels were associated with a significant reduction in the risk of all-cause mortality in MHD patients. The latter findings remained robust after adjustment for several important covariates including diabetes, dialysis vintage and serum IL-6 levels. Additionally, these observations were unique to 2-AG given that similar findings were not noted when evaluating the association of serum AEA levels, another major ECB ligand, with laboratory and clinical outcomes. In fact, higher serum AEA levels correlated with reduced concentrations of triglyceride-rich lipoproteins (VLDL) and increased serum concentrations of the pro-inflammatory cytokine, IL-6. In addition, higher serum AEA levels were not associated with improved survival and there was a trend toward worse outcomes in some of these analyses (Fig. 2).

While the exact mechanisms underlying these results remain to be elucidated, several aspects of these findings can be explained in the context of the mechanism of action of the ECB system and observational data from MHD patients. As mentioned earlier, there is abundant evidence linking overactivity of the ECB system to obesity and elevated triglyceride levels. While the latter findings may be associated with adverse outcomes in the general population, their association with outcomes is not as well-established in patients at high risk for PEW and cachexia such as the ESRD population. In fact, there is evidence that obesity and hypertriglyceridemia are associated with a lower risk of mortality in some MHD patients and these paradoxical associations may be partly related to the lower risk of cachexia in these patient subsets [8, 9, 10, 32]. Therefore, it can be speculated that in MHD patients, higher circulating levels of 2-AG and increased ECB system activity are associated with improved energy production and preservation. This manifests clinically with higher BMI and serum triglycerides, and reduced risk of cachexia and ultimately decreased risk of death (Fig. 3).

There are some mechanistic pathways that may support this theory. For instance, it has been demonstrated that CKD is associated with the wasting of adipose tissue and skeletal muscle through enhanced fat and protein catabolism [33]. This is partly attributed to increased expression of thermogenic genes in fat tissue and “browning” of white adipocytes resulting in increased thermogenesis and inefficient energy expenditure [15, 34]. In this regard, 2-AG-mediated activation of the ECB system has been shown to prevent expression of thermogenic genes and “browning” of white adipose tissue [35, 36]. Therefore, it is possible that increased serum 2-AG levels in ESRD prevent CKD-associated inefficient energy metabolism, which can increase the risk of cachexia and lead to poor outcomes. While the possibility that obesity and hypertriglyceridemia directly contribute to increasing serum 2-AG levels cannot be excluded, there is also evidence that activation of the EC system via 2-AG may play a causative role in elevated triglyceride levels [36]. This is mediated via activation of CB_1R, which leads to increased synthesis of fatty acids (through stimulation of the sterol regulatory element binding protein1c [SREBP1c] pathway) and decreased

fatty acid β-oxidation (downregulation of the peroxisome proliferator activated receptor-α pathway) [37]. Therefore, it is possible that increased energy production, in the form of fatty acids and elevated triglycerides, may be driven by higher 2-AG levels (Fig. 3). However, these notions remain speculative at this time and larger observational and mechanistic studies are needed to confirm these hypotheses and explore other potential pathways which can explain our findings.

It is interesting to note that while 2-AG and AEA are both considered key ligands of the ECB system that bind CB receptors with high affinity, the correlation of circulating levels of these lipid-derived mediators with laboratory and clinical indices and their association with all-cause mortality is considerably different. Similar findings have also been reported by other investigators and these differential effects have been partly explained in the context of functional selectively (also known as ligand bias) [38, 39, 40]. In this regard, it is well known that CB receptors are members of the G-protein-coupled receptors whose activation is associated with recruitment of various G-proteins and a series of downstream effects [41, 42]. However, there is growing evidence that the recruitment of these G-proteins is highly dependent and driven by the ligand which is activating the CB_1R, a concept known as functional selectivity [41, 42]. Hence, while 2-AG activation of CB receptors may be associated with recruitment of one set of mediators resulting in a given effect, activation by other mediators (such as tetrahydrocannabinol or AEA) can recruit a different group of molecules leading to a different outcome [42, 43]. For instance, it has been shown that changes in circulating levels of AEA are driven by hunger and respond to food in a manner consistent with homeostatic (hunger-driven) feeding. In contrast, the response of circulating 2-AG levels to food is driven by a meal's perceived hedonic value and 2-AG is involved in reward-driven feeding [44, 45, 46]. In a study similar to ours, Cote et al. [47] found that circulating 2-AG but not AEA levels correlated positively with BMI, fasting plasma triglycerides and negatively with HDL-C. It was argued that these differential correlations are supported by evidence that 2-AG is the more effective activator of ECB system [48]. Furthermore, it has been shown that AEA can bind to several other targets, thereby mediating differential effects [49]. Therefore, there is evidence that ECB ligands can facilitate differential effects under different conditions.

Several limitations of our study need to be acknowledged. The present findings should be qualified given the observational nature of our study design, and future mechanistic studies are needed to determine if a causal link between circulating 2-AG levels and reduced mortality exists. While another limitation of our study is the small sample size, it should be noted that this is one of the largest studies of circulating ECBs levels in a single patient cohort. Nevertheless, larger studies are needed to confirm and build on our findings. Although we adjusted for some potential confounders such as diabetes and inflammation (IL-6), given lack of data regarding hospitalization and infections, residual confounding cannot be excluded. Additionally, our patient cohort lacked detailed nutritional data (such as food intake) and information on visceral adipose tissue and therefore we cannot measure or rule out a potential impact from these factors in the associations observed in this study. Future studies will need to examine these important endpoints in the context of circulating ECBs in MHD patients. Finally, we used non-fasting serum for all analyses and hence cannot rule out the potential impact of food intake on the levels of circulating ECBs. It has been reported, however, that circulating levels of 2-AG are not affected by feeding [46] or in-

traduodenal administration of a lipid emulsion [50]. In addition, given that we used non-fasting serum for all of our analyses, variability of results based on fasting state of the patients is less likely.

In conclusion, we found that circulating levels of 2-AG, a major ECB ligand, may play an important role in determining body mass and the risk of mortality in ESRD patients treated with MHD. These observations were unique to 2-AG as similar findings were not obtained with serum AEA, the other major ligand of the ECB system. Future studies need to confirm these findings, investigate the mechanisms responsible for these associations, and examine the modulation of the ECB system (especially 2-AG) as a potential target for therapy in MHD patients.

Disclosure Statement

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

Author Contributions

H.M., D.P., and K.K.-Z. research idea and study design. H.M., C.P., E.S., A.S.Y., and C.M.R. data acquisition. H.M., D.A.A., C.P., and E.S. data analysis/interpretation. H.M., C.P., and E.S. statistical analysis. D.P., K.K.-Z., N.D.V., and N.V.D. supervision or mentorship. 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.

Supplementary Material

Supplementary data

Acknowledgments

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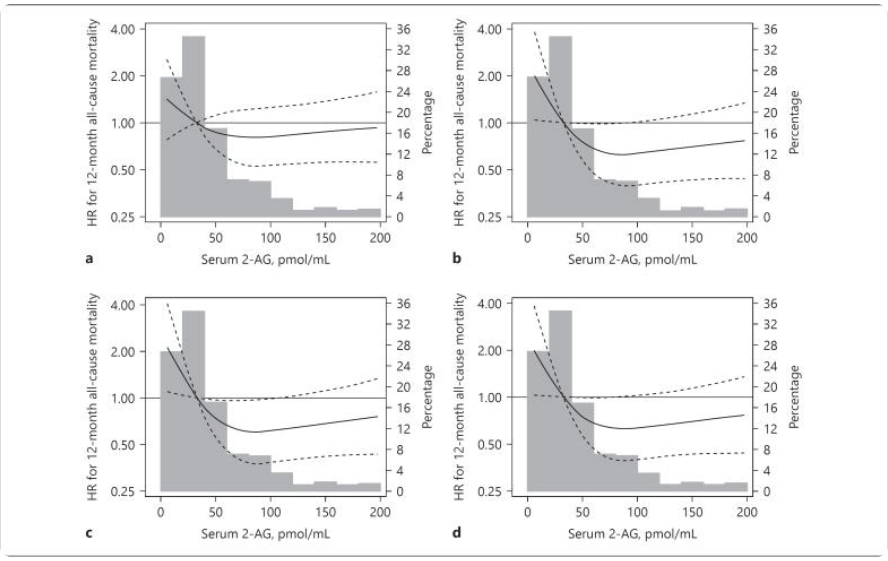
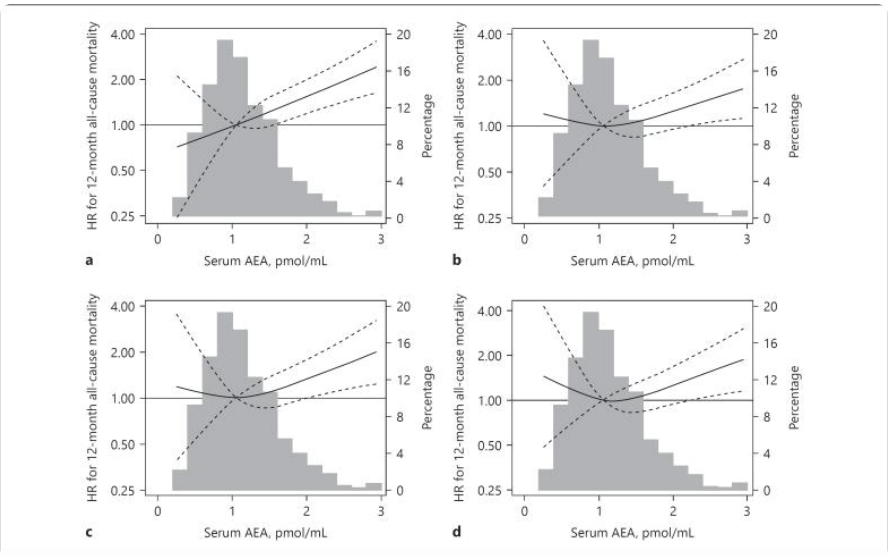
Figures and Tables

Fig. 1



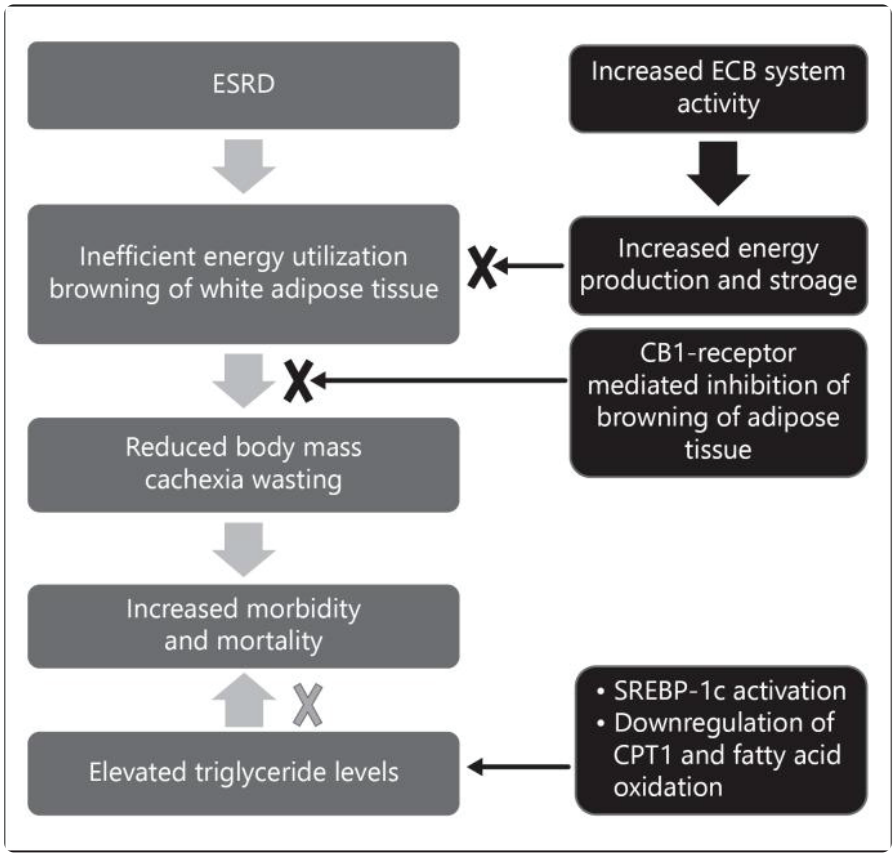
Restricted cubic splines of the association between serum 2-AG and 12-month all-cause mortality among 400 MHD patients. Splines were adjusted for covariates in models 1–4 as follows: (**a**) model 1 (unadjusted); (**b**) model 2 (age, gender, race and ethnicity); (**c**) model 3 (model 2 + diabetes and dialysis vintage); and (**d**) model 4 (model 3 + IL-6). Solid and dotted lines represent hazard ratios and 95% CIs respectively. HR, hazard ratio; 2-AG, arachidonoyl-*sn*–glycerol.

Fig. 2



Restricted cubic splines of the association between serum AEA and 12-month all-cause mortality among 436 MHD patients. Splines were adjusted for covariates in models 1–4 as follows: (**a**) model 1 (unadjusted); (**b**) model 2 (age, gender, race and ethnicity); (**c**) model 3 (model 2 + diabetes and dialysis vintage); and (**d**) model 4 (model 3 + IL-6). Solid and dotted lines represent hazard ratios and 95% CIs, respectively. HR, hazard ratio; AEA, anandamide.

Fig. 3



Potential impact of increased serum 2-AG levels in patients with ESRD on MHD. Elevated circulating levels of 2-AG and overactivity of the ECB system can be associated with reduced energy loss (decreased browning of white adipose tissue) and enhanced energy production (increased fatty acid and triglyceride generation). The latter effects can help reduce the risk of cachexia and mortality in ESRD patients on hemodialysis. CB-1, cannabinoid 1 receptor; CPT1, carnitine palmitoyl-transferase-1; ECB, endocannabinoid; ESRD, end-stage renal disease; SREBP-1c, sterol regulatory element binding.

Table 1

Baseline characteristics of MHD patients according to serum 2-AG and AEA categories

Variable	Serum 2-AG, pmol/mL				Serum AEA, pmol/mL			
	total	<32.5	≥32.5	Standardized differences	total	<1.06	≥1.06	standardized differences
n (%)	400	200 (50)	200 (50)		436	217 (50)	219 (50)	
Age, years	55±14	54±15	56±13	0.14	55±14	53±15	57±14	0.24
Gender, female	45	41	49	0.15	44	42	46	0.08
Race (%)								
White	63	66	61	−0.09	62	67	57	−0.20
African-American	29	24	33	0.20	30	22	38	0.37
Asian	7	9	6	−0.10	7	10	4	−0.26
Other	1	2	0	−0.20	1	1	1	−0.04
Hispanic ethnicity	56	62	50	−0.24	54	61	48	−0.26
Diabetes	54	52	56	0.09	54	49	59	0.20
Access type								
Tunneled catheter	5	4	6	0.09	5	3	7	0.17
Temporary catheter	2	1	3	0.17	2	1	2	0.11
AV graft	15	13	16	0.11	15	11	20	0.26
AV fistula	78	83	74	−0.21	78	85	71	−0.32
BMI, kg/m^2	27.7±6.7	26.5±6.2	28.8±6.9	0.35	27.7±6.5	27.5±5.9	28.0±7.0	0.07

Data are presented as percentages, mean ± SD or median (IQR), where appropriate.

Percentages may not add up to 100 as a result of rounding. Data are not shown for unknown access type (<1% of entire study cohort). Baseline characteristics were compared between 2-AG and AEA categories with standardized differences (≥2 considered statistically significant).

AV, arteriovenous; MHD, maintenance hemodialysis; AEA, anandamide; 2-AG, 2-arachidonoyl-*sn*−glycerol; BMI, body mass index; IQR, interquartile range.

Table 2

Unadjusted and adjusted Spearman's rank correlation coefficients (Rho) between serum 2-AG and AEA levels and laboratory and clinical measures in maintenance hemodialysis patients

Variable	Serum 2-AG, pmol/mL				Serum AEA, pmol/mL			
	unadjusted		adjusted*		unadjusted		adjusted*	
	rho	*p* value	rho	*p* value	rho	*p* value	rho	*p* value
Laboratory tests								
Albumin, g/dL	−0.02	0.73	0.03	0.58	−0.15	0.006	−0.07	0.21
Creatinine, mg/dL	0.03	0.59	0.07	0.21	−0.14	0.007	−0.12	0.02
Ferritin, ng/mL	0.03	0.59	0.03	0.57	−0.03	0.60	−0.03	0.54
TIBC, mg/dL	0.18	0.001	0.22	<0.0001	−0.05	0.37	−0.006	0.92
PTH, pg/mL	0.004	0.95	−0.01	0.83	−0.02	0.67	−0.02	0.76
Lipids								
VLDL-C, mg/dL	0.35	<0.0001	0.41	<0.0001	−0.23	<0.0001	−0.18	0.0002
Triglycerides, mg/dL	0.43	<0.0001	0.47	<0.0001	−0.14	0.003	−0.13	0.009
Cholesterol, mg/dL	0.13	0.01	0.15	0.004	0.02	0.74	0.002	0.97
HDL-C, mg/dL	−0.29	<0.0001	−0.34	<0.0001	0.10	0.04	0.07	0.15
LDL-C, mg/dL	0.08	0.11	0.12	0.02	0.04	0.43	0.04	0.37
LPA-C, mg/dL	−0.02	0.65	−0.10	0.04	0.10	0.05	−0.006	0.91
NHDL, mg/dL	0.23	<0.0001	0.27	<0.0001	−0.04	0.36	−0.04	0.37
IL-6, pg/mL	−0.03	0.63	−0.06	0.32	0.19	0.0005	0.18	0.001
BMI, kg/m^2	0.19	0.0002	0.18	0.0005	0.05	0.28	−0.005	0.92
Body anthropometry measures								
Biceps skin fold, mm	0.22	<0.0001	0.23	<0.0001	−0.05	0.35	−0.06	0.28
Triceps skin fold, mm	0.21	0.0001	0.22	0.0002	−0.06	0.30	−0.09	0.13
Mid-arm muscle circ., mm	−0.007	0.91	0.002	0.97	0.09	0.10	0.05	0.33
Mid-arm circ., mm	0.15	0.007	0.15	0.008	0.07	0.22	0.02	0.74
NIR body fat, %	0.17	0.002	0.16	0.006	−0.01	0.85	−0.05	0.33

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

2-AG, 2-arachidonoyl-sn-glycerol; AEA, anandamide; 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; PTH, parathyroid hormone; TIBC, total iron-binding capacity; VLDL-C, very low-density lipoprotein-cholesterol; BMI, body mass index.