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Circulating endocannabinoid concentrations in grieving adults

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ABSTRACT

Bereavement is one of the most intense, distressing, and traumatic events an elderly person will experience. The symptom responses to bereavement vary, particularly during the first year. However, the neurobiology underlying the symptom variance in grief is poorly understood. The endocannabinoid signaling (ECS) system is stress-responsive; mounting evidence implicates the central ECS in psychopathology. The current study aimed to investigate the hypothesis that the ECS is abnormal in grief, using circulating eCB concentrations as a biomarker of central ECS. A predominantly older sample of grief participants, within 13 months following the death of a loved one, and healthy comparison (HC) participants were studied. Associations of circulating eCBs with symptom variance in grievers were also examined. A total of 61 (grief: n = 44; HC: n = 17) adults completed cross-sectional clinical assessments and a fasting blood draw. Assessments included the Inventory of Complicated Grief scale; the 17-item Hamilton Depression Rating Scale; and the Hamilton Anxiety scale. Serum eCB concentrations (i.e., *N*-arachidonoylethanolamine [AEA] and 2-arachidonoylglycerol [2-AG]) were quantified using isotope dilution, liquid chromatography-mass spectrometry. Relative to HC participants, grievers had significantly elevated serum AEA but similar 2-AG concentrations. In grievers, serum AEA concentrations were positively associated with depressive and anxiety symptoms, but only in those with low grief symptoms. These novel findings indicate that elevated circulating eCB concentrations are found following bereavement. The eCB signaling response varies based on the degree of grief severity. Circulating eCB measures may have the potential to serve as biomarkers of prolonged grief disorder.

1. Introduction

Bereavement is a trauma commonly experienced by older adults; more than 70% of the elderly experience loss of a loved one over a 30 month period (Williams et al., 2007). Such an attachment loss typically leads to acute grief symptoms that include yearning and longing for the loved one; intrusive thoughts and avoidance of reminders of the deceased; sadness and other painful emotions; loneliness, shock and disbelief; and emotional numbness (Shear, 2012; Shear et al., 2013; Simon, 2013). Bereavement among the elderly is associated with precipitating or worsening of mental disorders; decrements in physical health; and increased risk of hospitalization; cardiovascular risk; and premature mortality (Aalbaek et al., 2017; Ennis and Majid, 2019; Stroebe et al., 2007). Most individuals successfully adapt to the emotional consequences of bereavement without seeking mental health care. However, in about 10%, acute grief becomes protracted and debilitating, leading to the development of prolonged grief disorder (PGD) or complicated grief (Shear, 2012; Simon, 2013), a clinically diagnosable mental health condition.

Despite our understanding that grief responses vary during the first year following an attachment loss, the neurobiology underlying this symptom variance is poorly understood. Current known risk factors for PGD include female gender, old age, insecure attachments in early life, death of a child or life partner, unexpected or violent death of a loved one, a history of mood and anxiety disorder, past trauma or loss, and social circumstances (e.g., low socioeconomic status, poor social support, etc.), among others (Shear, 2012; Simon, 2013). While these factors provide general information regarding who might be at risk for

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PGD, biomarkers could provide a more sensitive and specific identification method. More importantly, a better understanding of the biological determinants contributing to PGD in older adults could lead to targeting interventions to at-risk individuals and the development of novel treatments. Research on the biological determinants of PGD is in its infancy; we are exploring the role of the endocannabinoid signaling (ECS) system based upon previous studies indicating a role for the central nervous system (CNS) ECS in emotion regulation.

The ECS system has a neuromodulatory role in the regulation of multiple physiological functions, including regulation of mood (Hill et al., 2009a). The ECS system is comprised of the cannabinoid receptors (CB1 and CB2 receptors); their lipid ligands (the endocannabinoids [eCBs] *N*-arachidonoylethanolamine [AEA or anandamide] and 2-arachidonoylglycerol [2-AG]); and the synthetic and degradative enzymes for the eCBs (Hill et al., 2009a). The ECS system is altered by stress and regulates stress responsivity, fear learning, and emotionally driven behavior (Hill and Gorzalka, 2009; Hillard, 2014; Morena et al., 2016). The eCB signaling inhibits stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis and enhances recovery to basal activity following stress cessation (Hill et al., 2011; Micale and Drago, 2018). Preclinical data indicate that enhanced eCB mediated signaling in the amygdala promotes resilience to adverse effects of acute traumatic stress and facilitates adaptation to stress exposure (Bluett et al., 2017). There is evidence that chronic stress-induced reductions in the ECS system produce neurochemical and behavioral changes in rodents that mimic those seen in human major depression (Hill et al., 2008a, 2005; Wang et al., 2010). Mounting evidence implicates the ECS system in the pathophysiology of various psychiatric conditions in humans, including major depression, anxiety, and trauma-related disorders (Hill and Gorzalka, 2009; Hill et al., 2009a; Hillard, 2018). Since bereavement is one of the most intense, emotionally distressing and traumatic events an individual can experience, we hypothesized that ECS system measures could have mechanistic value in explaining the symptom variance in older grieving adults during the first-year post-loss.

Circulating concentrations of endocannabinoids AEA and 2-AG are considered surrogate measures of the central ECS system and can be reliably measured in human serum and plasma (Hillard, 2018; Lam et al., 2010). Many correlational studies have been carried out to examine the relationships between circulating concentrations of the eCBs and various parameters of mood and anxiety symptoms in healthy subjects and patient populations, including in those with major depression and post-traumatic stress disorder (PTSD) (Hillard, 2018). There is fairly good agreement in the literature that low circulating 2-AG concentrations are associated with depression (Hill et al., 2009b, 2008b). This is consistent with the preclinical data that low 2-AG/CB1R signaling induces depressive-like symptoms (Hill and Gorzalka, 2009; Hill et al., 2009a). The data regarding relationships of circulating AEA concentrations and measures of mood and anxiety, however, are less consistent. For example, women with diagnosed depression exhibit negative correlations between circulating AEA concentrations and both cognitive and somatic anxiety (Hill et al., 2008b), and healthy young adults demonstrate negative correlations of circulating AEA with anxiety (Dlugos et al., 2012). On the other hand, evidence from other depression studies are mixed (Belitardo de Oliveira et al., 2019; Hill et al., 2008b; Romero-Sanchiz et al., 2019; Stensson et al., 2018), suggesting that the circulating AEA-mood relationships are complex. Given that bereavement is a common traumatic experience in older individuals and is accompanied by emotion dysregulation, we hypothesized that grieving individuals will exhibit abnormal circulating eCB concentrations compared to healthy comparison (HC) participants.

To test this hypothesis, the objectives of this pilot study were to examine circulating eCB concentrations in grievers, relative to HC participants. We further examined whether there are relationships between circulating eCB concentrations and clinical measures of grief, depressive, and anxiety symptoms in grievers. Finally, we explored whether the associations between circulating endocannabinoid concentrations and depressive and anxiety symptoms would be moderated by grief severity.

2. Material and methods

2.1. Study participants

2.1.1. Recruitment

A total of sixty-one adults, aged 50 years and older, were recruited from the community. Forty-four grief participants, who were within 13 months following the death of a loved one, were recruited via advertisements, and through referrals from grief groups and hospice counselors. The remaining seventeen were HC participants who were recruited from the community through advertisements. All participants provided written informed consent according to our Institutional Review Board-approved protocols.

2.1.2. Assessment procedures

During the baseline clinical visit, all participants completed comprehensive clinical assessments, including a Structured Clinical Interview for DSM-5 Research Version. Sociodemographic characteristics, medical and psychiatric histories, and medication history were obtained, and a neurological examination was performed. All participants also completed self-report questionnaires and a battery of tests. These included the 17-item Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1980), a 30-item Yesavage Geriatric Depression Scale (GDS) (Yesavage et al., 1982), Hamilton Anxiety Scale (HAM-A) (Maier et al., 1988), Cumulative Illness Rating Scale-Geriatric version (CIRS-G) (Miller et al., 1992), modified Hachinski Ischemic Scale (HIS) (Hachinski et al., 1975), Scale of Suicidal Ideation (SSI) (Beck et al., 1979), Mini Mental State Exam (MMSE) (Folstein et al., 1975) and Mattis Dementia Rating Scale-2 (Lucas et al., 1998). All grief participants also completed the Inventory of Complicated Grief (ICG) (Prigerson et al., 1995), and information related to the relationship of deceased and time since loss (TSL) were documented.

A fasting blood draw was obtained within 19 ± 14 days of the clinical visit in which the assessments were carried out (grief group: 20 ± 15; HC: 16 ± 12).

2.1.3. Inclusion and exclusion criteria

All participants had to be proficient in English and have adequate visual and auditory acuity, a score $\leq$ 4 on the HIS, and a score $\geq$ 24 on the MMSE.

Exclusion criteria included a lifetime history of bipolar or psychotic disorders; alcohol or substance use disorders during the past five years; acute suicidality (assessed using the SSI and the third HAM-D item score or judged by a clinician to be at serious suicide risk); a history of neurological illnesses, including seizures, stroke, dementia of any etiology, severe head injury, brain tumor, etc.; and delirium/unstable medical conditions determined using the CIRS-G score of 4 in any category.

Participants were included in the grief group if they were within 13 months following the death of a loved one. Grief participants who met current DSM-5 criteria for depressive, anxiety, or trauma- and stressor-related disorders were not excluded if the onset followed bereavement. Since this was an observational study, grief participants could be on antidepressant medications and/or low doses of benzodiazepines or gabapentin as long as the doses remained stable for at least four weeks before clinical and fasting blood draw visits.

Inclusion/exclusion criteria for HC participants were identical to those for grief participants, except for the following differences: No lifetime history of any psychiatric illnesses; no history of death of a spouse/partner or a first-degree relative within 18-months of enrollment; an HDRS score <7 and/or a GDS score <8; and no psychotropic medications.

2.2. Experimental procedure

2.2.1. Circulating eCB concentrations

For measurement of circulating eCB concentrations (independent variables), the fasting blood draw was conducted in all but one participant between 7:00 a.m. and 11:00 a.m. One remaining participant had a fasting blood draw before 12:30 p.m. Blood was refrigerated after collection and serum was separated by centrifugation within 60 min and stored in 3 mL aliquots at −80 °C. Serum concentrations of AEA and 2-AG were determined in extracted lipids from serum samples using stable isotope-dilution, liquid chromatography-mass spectrometry quantification methods as described previously (Crombie et al., 2018).

2.2.2. Clinical outcome measures

Clinical measures of interest, ICG, HAM-D, and HAM-A, were administered. ICG is a 19-item, self-report questionnaire that has good to excellent psychometric properties and assesses symptoms of PGD. ICG has been previously utilized in PGD treatment studies, and the total scores range from 0 to 76; a score of 30 or higher is indicative of PGD (Prigerson et al., 1995). The 17-item HAM-D (Hamilton, 1980) and 14-item HAM-A (Maier et al., 1988) are widely used, validated, clinician-administered instruments that measure symptoms of depression and anxiety respectively. Each item score is summed to generate a total score ranging from 0 to 52 for HAM-D and from 0 to 56 for HAM-A.

2.3. Statistical analyses

Demographics and clinical characteristics were compared between grief and HC participants using Mann-Whitney tests; chi-square tests were used to examine for gender and race differences.

Mann-Whitney tests were used to compare the AEA and 2-AG concentrations between grief and HC groups. To examine the associations between eCB concentrations (independent variables) and clinical variables (dependent measures), linear regression models were used, after adjusting for age, gender, and TSL. Further, grief participants were dichotomized into two groups based on their ICG scores: high (i.e., ICG > 30) and low (i.e., ICG ≤ 30) symptom groups. Then, linear regression models were repeated to explore if the associations of eCBs with depressive and anxiety symptoms were moderated by grief symptom severity while adjusting for age, gender, and TSL. The overall significance was set at $p < 0.05$ (two-tailed) for each of our linear regression analyses. All analyses were conducted using IBM SPSS Statistics Version 26.0 (IBM, Armonk, New York).

3. Results

3.1. Demographic and clinical characteristics

The demographic data for the study is shown in Table 1. Compared with HC participants, grievers were significantly younger and, as expected, had significantly higher depressive and anxiety symptom scores. The groups did not differ on any other demographic measure. In the grief group, the mean ICG score was 29.0 ± 15.7 and time since loss (TSL) of the loved one was 160.1 ± 91.6 days. Twenty-five of the 44 grief participants had an ICG score of > 30. Twenty-six (59%) grief participants had lost a spouse/life partner, or child, and fourteen (32%) had experienced the death of a parent or sibling. Twenty-three grief participants met DSM-5 criteria for a bereavement-related psychiatric disorder (major depressive disorder only: n = 18; unspecified depressive disorder: n = 1; unspecified anxiety disorder: n = 1; co-occurring major depressive disorder and unspecified anxiety disorder: n = 2; and concurrent major depression and post-traumatic stress disorder: n = 1). Thirteen grief participants were on antidepressants (monotherapy: n = 7; combination therapy: n = 6). TSL was marginally associated with HAM-D (partial r = 0.30; p = 0.06) but not with ICG (partial r = 0.10; p = 0.51) or HAM-A (partial r = 0.22; p = 0.15) scores.

Table 1
Baseline demographics and clinical characteristics.

Variable	Healthy Comparison (n = 17)	Grief (n = 44)	Test statistic[a]	*p*
Age – *Mean (SD)*	71.4 (7.9)	65.8 (9.2)	2.27	.02
Gender – *n (%)*			1.59	.21
Female	14 (82%)	27 (61%)		
Male	3 (18%)	17 (39%)		
Race – *n (%)*			.01	.91
White	15 (88%)	41 (93%)		
Black	2 (12%)	3 (7%)		
Years of education - *Mean (SD)*	15.3 (2.2)	15.6 (3.6)	.06	.95
GDS – *Mean (SD)*	2.4 (2.6)	12.2 (9.2)	3.65	<.01
HAM-D – *Mean (SD)* [b]	2.9 (1.6)	12.2 (8.0)	3.71	<.01
HAM-A – *Mean (SD)*	2.3 (1.8)	7.8 (5.1)	3.78	<.01
ICG – *Mean (SD)*		29.0 (15.7)		
MMSE – *Mean (SD)*	28.7 (1.4)	28.4 (1.6)	.73	.47
Body mass index – *Mean (SD)* [c]	29.3 (5.9)	29.3 (4.8)	.13	.90
Time since loss (days) – *Mean (SD)*		160.1 (91.6)		

GDS: 30-item Yesavage Geriatric Depression Scale; HDRS: 17-item Hamilton Depression Rating Scale; HAM-A: Hamilton Anxiety Scale; ICG: Inventory of Complicated Grief; MMSE: Mini-Mental State Exam.

[a] For categorical data, statistical significance was evaluated using Chi-square test for dichotomous related variables. For continuous variables, significance was evaluated by Mann-Whitney Z-test.

[b] Three healthy comparison participants had missing HAM-D scores.

[c] Two grief participants had missing body mass index values.

3.2. Circulating eCB differences, and their relationships with clinical measures

Grievers, compared with HC participants, had significantly elevated serum AEA ($p = 0.001$) (Fig. 1a) but similar 2-AG ($p = 0.80$) (Fig. 1b) concentrations. TSL was not significantly associated with AEA (partial $r = 0.01$; $p = 0.97$) or 2-AG (partial $r = -0.11$; $p = 0.49$) concentrations.

In the grief group, linear regression revealed that, after adjusting for age, gender and TSL, serum AEA concentrations were positively associated with HAM-D (partial $r = 0.45$, $p < 0.01$; Fig. 2a) and HAM-A (partial $r = 0.56$, $p < 0.01$; Fig. 2b) scores. The relationship between serum AEA concentrations and ICG was marginally significant (partial $r = 0.31$, $p = 0.05$) (Fig. 2c). No significant associations between 2-AG concentrations and clinical measures were found in the grief group. Similarly, serum eCB concentrations did not correlate with clinical measures in the HC group.

To further explore if the AEA–clinical symptom associations were moderated by grief symptom severity, we separated grievers into high (i.e., ICG score > 30) and low ICG (i.e., ICG score ≤ 30) groups. The subsequent linear regression analyses showed that the relationships of AEA with HAM-D (Fig. 3a) and HAM-A (Fig. 3b) measures were significant in the low ICG group (HAM-D: partial $r = 0.48$, $p = 0.04$; HAM-A: partial $r = 0.66$, $p < 0.01$), but not in those with high ICG scores (HAM-D: partial $r = 0.24$, $p = 0.24$; HAM-A: partial $r = 0.35$, $p = 0.09$).

4. Discussion

This pilot study revealed that serum AEA concentrations were significantly elevated in a predominantly older sample of grieving participants compared with their healthy counterparts. Circulating 2-AG concentrations were similar in both groups. Serum AEA concentrations were positively associated with depressive and anxiety symptoms in grievers. In particular, the positive association of AEA with depressive

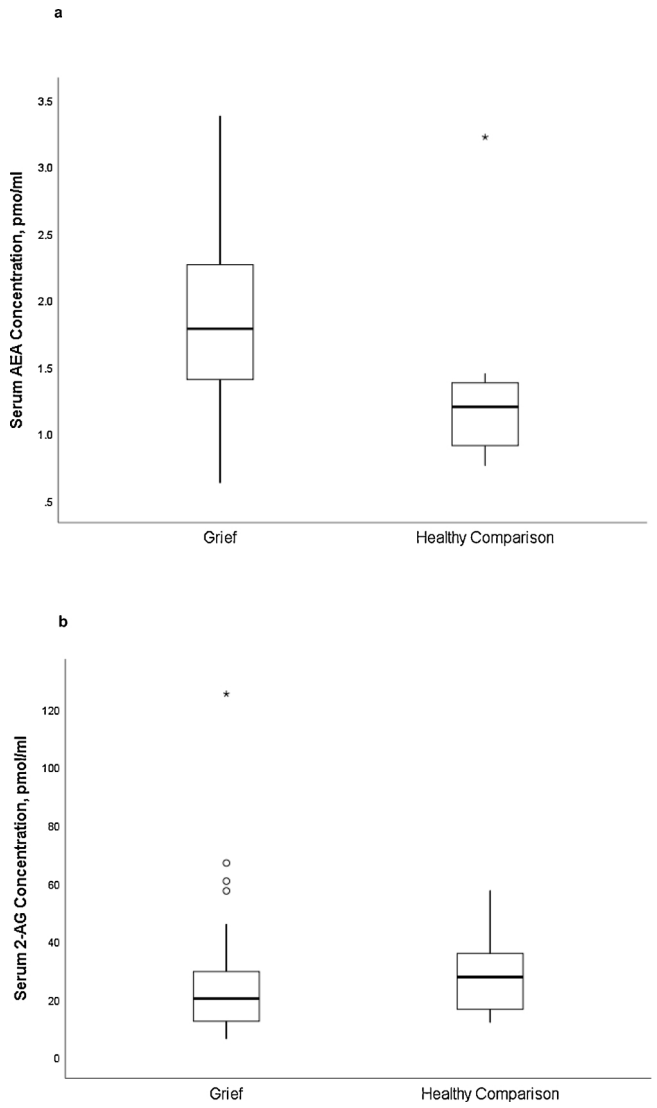

Fig. 1. Circulating endocannabinoid concentration differences between grief and healthy comparison participants. Illustration of serum (a) AEA and (b) 2-AG group differences. In the boxplot, data indicated by circles and asterisks are 1.5 and 3.0 interquartile range beyond the third quartile, respectively. Abbreviations. AEA: *N*-arachidonoylethanolamine or anandamide; 2-AG: 2-arachidonoylglycerol.

and anxiety symptoms was seen in those with low, but not high, grief symptoms after adjusting for age, gender, and time since loss. Our findings demonstrate that, like other mood and trauma-related disorders (Hillard, 2018), bereavement, a commonly encountered trauma in older adults, is accompanied by abnormal circulating eCB concentrations that correlate with clinical symptoms.

Our finding that circulating AEA concentrations are elevated in grievers is consistent with the results of some previous studies in healthy and depressed individuals. An acute psychosocial stress-induced increase in circulating AEA concentrations is found in healthy individuals (Dlugos et al., 2012). A study of healthy but physically inactive individuals also showed that manipulation of circulating AEA concentrations with an exercise program revealed positive correlations between AEA and mood disturbances (Belitardo de Oliveira et al., 2019). Several studies have found that circulating AEA concentrations are higher in depressed individuals (Hill et al., 2008b; Romero-Sanchiz et al., 2019), although another study found serum AEA concentrations are reduced in major depression (Hill et al., 2009b). On the other hand, the relationship between circulating AEA and symptoms of anxiety in trauma-related conditions is equivocal. Compared to individuals without PTSD, AEA concentrations were diminished in noncombat-related PTSD (Neumeister et al., 2013), unchanged in those with PTSD from exposure to World Trade Center attacks (Hill et al., 2013) and childhood sexual abuse (Schaefer et al., 2014), and increased in war refugees (Hauer et al., 2013).

Some insights into the causal relationship between circulating AEA concentrations and depressive and anxiety symptoms can be found in studies in which acute changes in AEA concentrations are examined together with changes in mood or anxiety. For example, moderate- to high-intensity exercise-induced increases in AEA concentrations are associated with positive affect in healthy individuals (Raichlen et al., 2012, 2013). Increases in AEA after moderate intensity exercise are also associated with improvements in depressive and anxiety symptoms in women with major depression (Meyer et al., 2019). Interestingly, while exercise-induced increases in circulating 2-AG concentrations are lost in individuals with PTSD, elevations in AEA are preserved (Crombie et al., 2019). These findings, together with preclinical data that AEA-mediated signaling in the brain is associated with reduced anxiety and elevated mood (Hill and Gorzalka, 2009; Hill et al., 2009a; Hillard, 2014; Morena et al., 2016), suggest the hypothesis that circulating AEA concentrations are mechanistically associated with improved mood and reduced anxiety.

However, in the present study, circulating AEA concentrations are associated with increased anxiety and depressive symptoms. As was suggested in an earlier report (Hill et al., 2008b), we hypothesize that these relationships reflect compensatory recruitment of the ECS system in an attempt to maintain elevated mood, reduced anxiety, and low overall grief severity in the initial months following an attachment loss. In other words, we hypothesize that an engaged ECS system response, as evidenced by elevated serum AEA concentrations, reflects an attempt to buffer against a negative stress response in individuals with grief. We further hypothesize that effective AEA-mediated signaling, evoked by the stresses of attachment loss and feelings of depression and anxiety, accelerates successful adaptation and transition to integrated grief in the elderly. Our data that the positive association between serum AEA concentrations and depressive and anxiety symptoms were only found in those with low grief symptoms support this hypothesis.

An alternative explanation is that AEA-mediated signaling may respond differently in grievers at low- versus high-risk for developing PGD. This is in agreement with earlier investigations that have examined the distinct effects of stress exposure on the eCB activity in high-risk and diseased individuals relative to low-risk groups. Exposure to parabolic flight maneuver-related stress, for instance, resulted in circulating AEA increases in healthy individuals who did not develop motion sickness, but an impaired eCB response in those who did (Chouker et al., 2010). Our results, therefore, lead us to postulate that an attachment loss induced enhanced peripheral AEA response results in transient increases in depressive and anxiety symptoms. This may signal an adaptive ECS system function, which is vital to keep grief symptoms at low levels during the acute grief phase, maintain homeostasis, and hasten the transition to integrated grief. However, in some grievers, a blunted peripheral AEA response may contribute to intense and disruptive symptoms during the acute phase, and persistent and protracted symptoms over time, ultimately leading to the development of PGD. Longitudinal studies are needed to explore this hypothesis.

This preliminary investigation has several limitations that should be considered when interpreting the results reported here. Our study had a small sample size, and therefore, our data are primarily for hypothesis-generating purposes. Since our data are cross-sectional, we caution against inferring causality. To validate the eCB hypothesis in grief, our findings require replication in a longitudinal study comprising of an adequately powered grief sample. In our grief group, over one-half met DSM-5 criteria for a bereavement-related psychiatric disorder; a majority had depressive and/or anxiety disorders. These data are consistent with epidemiological studies that have reported a significant

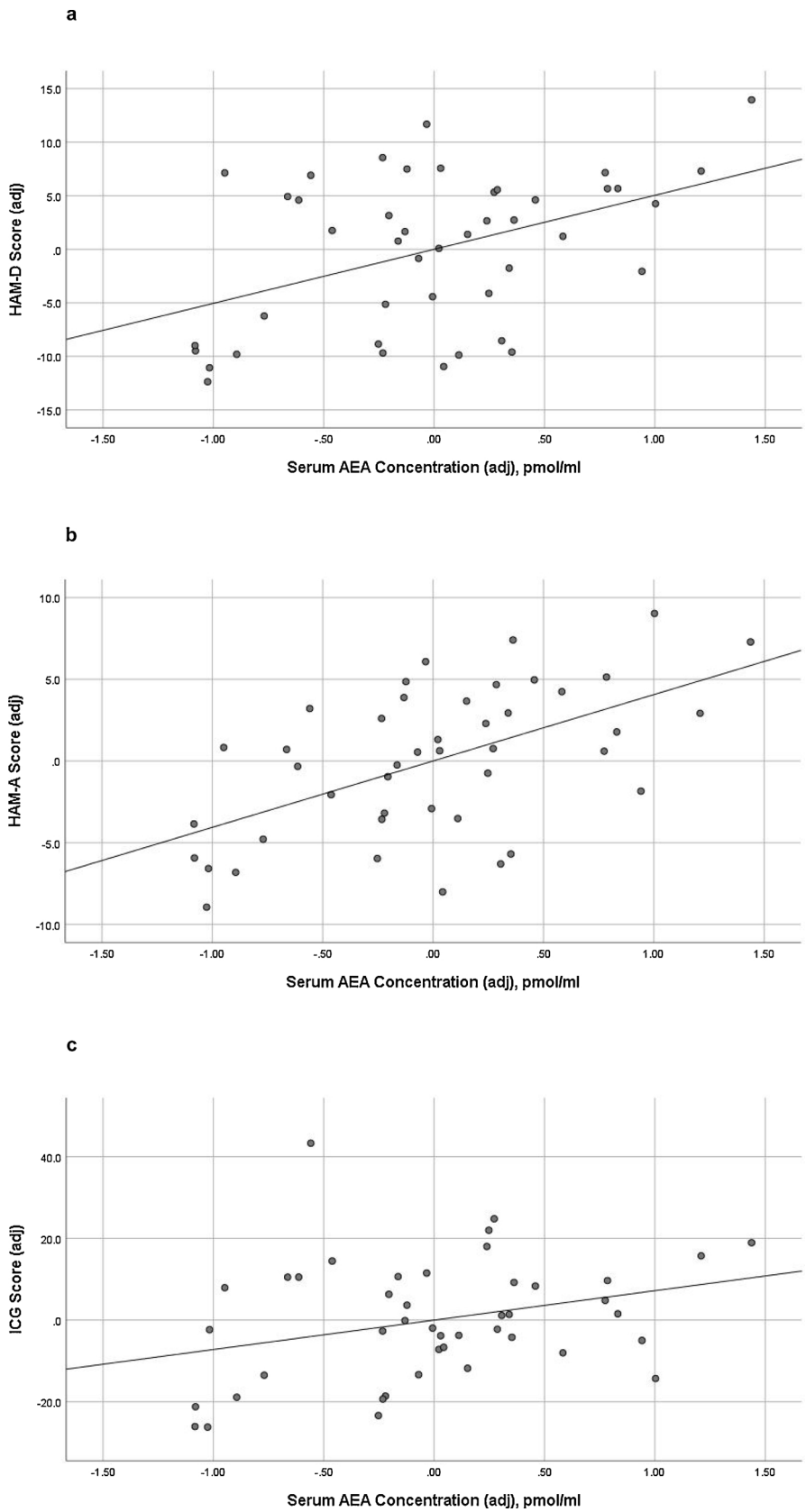

Fig. 2. Relationship between serum AEA concentrations and multidomain clinical variables. Illustration of age, gender, and time since bereavement adjusted (a) HAM-D, (b) HAM-A, and (c) ICG scores in grief. Fig. 2a–c show the residuals after adjusting for age, gender, and time since bereavement and the linear regression line. Abbreviations. adj: adjusted; AEA: *N*-arachidonoylethanolamine or anandamide; HAM-D: 17-item Hamilton Depression Rating Scale; HAM-A: 14-item Hamilton Anxiety Scale; ICG: Inventory of Complicated Grief Scale.

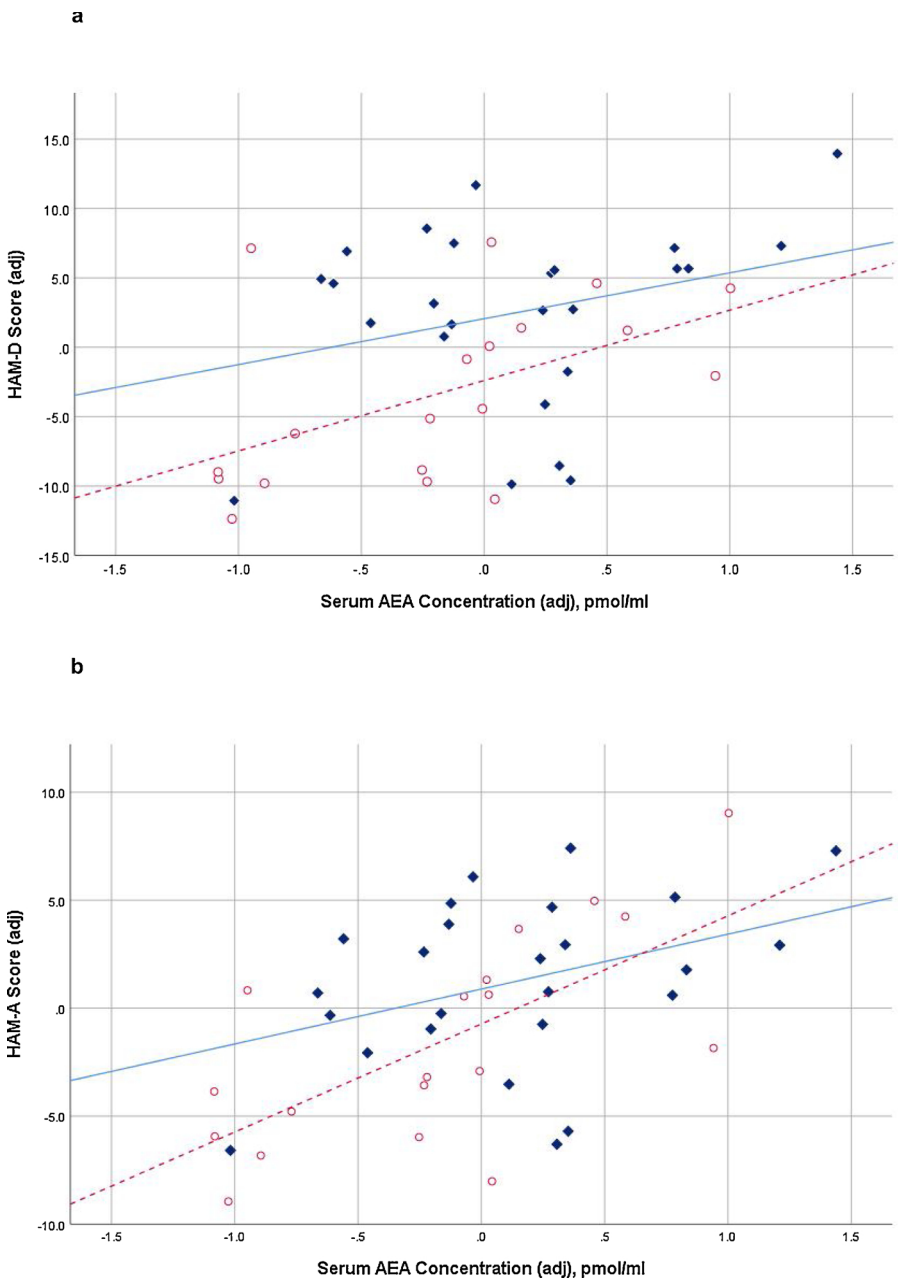

Fig. 3. Relationship of serum AEA concentrations with (a) depressive and (b) anxiety symptoms according to the degree of grief symptom severity. Fig. 3a,b show the residuals after adjusting for age, gender, and time since bereavement and the linear regression line for each group. The high (i.e., ICG > 30) and low (i.e., ICG ≤ 30) grief symptom groups are depicted by solid blue and dashed red lines respectively. Participants included in the high and low grief groups are presented as filled blue diamond and open red circles respectively. Abbreviations. adj: adjusted; AEA: *N*-arachidonoylethanolamine or anandamide; HAM-D: 17-item Hamilton Depression Rating Scale; HAM-A: 14-item Hamilton Anxiety Scale; ICG: Inventory of Complicated Grief Scale (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

number of acutely grieving individuals meet criteria for a psychiatric condition during the first year following bereavement (Shear, 2012; Simon, 2013; Zisook et al., 2012), though PGD, according to the DSM-5 for persistent complex bereavement disorder, can only be diagnosed 12 months following the death of a loved one. Future studies should examine the variance in circulating eCB concentrations in different bereavement-related psychiatric conditions. Also, multivariate approaches could be used to parse out the associations of circulating eCB concentrations with multidimensional symptoms that characterize grief in its acute phase. Additionally, 30% of grievers were on antidepressants. Thus, the influence of antidepressant use on the circulating eCB findings in grief should be examined in future studies, particularly in light of recent data that antidepressant use alters circulating 2-AG concentrations (Romero-Sanchiz et al., 2019). We did not collect information on early life adversities and a history of losses in our study cohort. Due to the small sample size, we were also unable to examine the associations of relationship to the deceased and nature of the death (e.g., violent death from suicide or homicide) on circulating eCB concentrations in our grief sample. Since the circulating eCB responses may be more pronounced immediately following stress exposure, future investigations should enroll participants who lost their loved one closer to the time of the study. Although the clinical and blood draw occurred on separate days, the time between visits were similar in the grief and HC groups ($p = 0.22$). Since the clinical response following bereavement lasts for months in a grieving individual, we believe that the circulating eCBs are likely related to clinical symptoms, even though they weren't measured at the same time. Future investigations should make attempts to complete eCB measurements on the day of clinical assessments. The fasting blood draws for all but one of the participants in this study were completed between 7 and 11 a.m.. Although there is evidence that circulating 2-AG concentrations rise significantly during this time period (Hanlon et al., 2015), the concentrations of AEA do not change very significantly, except following the morning meal (Hanlon, 2020). Nevertheless, future studies should either decrease the time range for blood draws across participants or include the time of blood draw as a covariate in statistical models.

5. Conclusions

Our data provide novel evidence demonstrating that circulating AEA concentrations are significantly elevated in older individuals following an attachment loss. These results lead us to hypothesize that elevated AEA concentrations as a consequence of bereavement, a stressful and traumatic experience, represent an efficient and normal functioning CNS ECS system. We hypothesize that a blunted AEA response may increase the likelihood of poor adaptation and future development of PGD. Future longitudinal studies that enroll a larger sample of elderly participants are essential to further investigate these hypotheses. Such studies could aid in the comprehensive understanding of the biomarker potential of circulating eCB measures in PGD. Future investigations should also examine the role of brain neuronal systems implicated in emotion regulation in explaining the associations between peripheral ECS system measures and clinical symptomatology characterizing grief.

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Author Contributions

Harfmann E, Larson ER, Hillard CJ, Goveas JS: Conception and design

Claesges SA, Goveas JS, Sauber, G: Acquisition of data

Harfmann E, McAuliffe T, Larson ES, Sauber G, Hillard CJ, Goveas JS: Analysis or interpretation of data

Harfmann E, Larson ER, Hillard CJ, Goveas JS: Drafting the original version

Harfmann E, McAuliffe T, Larson ER, Claesges SA, Sauber G, Hillard CJ, Goveas JS: Revising and editing the manuscript critically for important intellectual content, and gave final approval

Meeting presentation

This work was accepted as an abstract for presenting at the ICRS2020 30th International Cannabinoid Research Society Symposium on the Cannabinoids, Galway, Ireland, July 4–9, 2020 (meeting was canceled due to the COVID-19 pandemic).

Declaration of Competing Interest

None.

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