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ORIGINAL ARTICLE

Elevated ratio of serum anandamide to arachidonic acid intake in community-dwelling women with high depressive symptoms

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Key words: **Anandamide; Arachidonic acid; 2-arachidonoylglycerol; Depression; Docosahexaenoic acid (DHA); Eicosapentaenoic acid (EPA); Endocannabinoids; Female population; Polyunsaturated fatty acids**

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Abstract

OBJECTIVES: The purpose of the present study was to investigate the serum levels of endocannabinoids (eCBs; anandamide: AEA and 2-arachidonoylglycerol: 2-AG) and daily intake of polyunsaturated fatty acids (PUFAs; arachidonic acid: ARA, docosahexaenoic acid: DHA, and eicosapentaenoic acid: EPA) among subjects with high and low depressive symptoms.

METHODS: The participants comprised female community-dwellers aged 40 years or older in Japan. Among 208 females, fourteen participants with high depressive symptoms and ten participants with low depressive symptoms were selected for this study. The depressive symptoms were measured by the Japanese version of the Centre for Epidemiologic Studies Depression Scale (CES-D). The daily intake of PUFAs were assessed utilising the brief-type self-administered diet history questionnaire. The blood samples were analysed for AEA, 2-AG, and the CB receptor 1 gene (*CNR1*) single nucleotide polymorphism (SNP) rs806377.

RESULTS: The ratio of AEA serum level to ARA intake (AEA/ARA) in high depressive participants was significantly higher compared with those in low depressive participants even after controlling for confounders, whereas there were no significant differences in the serum concentrations of eCBs, daily intake of PUFAs, as well as the *CNR1* SNP (rs806377) between the high and low CES-D scored groups.

CONCLUSION: The elevated level of AEA/ARA among high depressive participants suggests that the conversion rate of ARA to AEA may be accelerated in depressive individuals.

Abbreviations:

eCBs	- endocannabinoids
CNS	- central nervous systems
2-AG	- 2-arachidonoylglycerol
AEA	- arachidonoylethanolamide (anandamide)
ARA	- arachidonic acid
BDHQ.	- brief-type self-administered diet history questionnaire
BDI	- Beck Depression Inventory
BMI	- body mass index
CB1R	- endogenous cannabinoid receptors-1
CB2R	- endogenous cannabinoid receptors-2
CNR1	- CB1R gene
COVID-19	- coronavirus disease 2019
DHA	- docosahexaenoic acid
DHEA	- ethanolamide derivatives docosahexaenoyl ethanolamine
DHQ	- diet history questionnaire
EPA	- eicosapentaenoic acid
EPEA	- eicosapentaenoyl ethanol-amide
FAAH	- fatty acid amide hydrolase
MAGL	- mono-acylglycerol lipase
MDD	- major depressive disorder
PUFAs	- polyunsaturated long-chain fatty acids
QC	- quality control
S.D.	- standard deviation
S.E.	- standard error
SNP	- single nucleotide polymorphism

INTRODUCTION

The endocannabinoids (eCBs) are endogenous ligands for cannabinoid receptors and the eCB system is implicated in various psychosomatic processes such as depression, anxiety, chronic pain, and obesity (Chanda *et al.* 2019). The eCB system is a neuromodulator comprised of two endogenous cannabinoid receptors-1 (CB1R) and -2 (CB2R), as well as their two primary endogenous ligands, arachidonoylethanolamide (AEA; a.k.a. anandamide) and 2-arachidonoylglycerol (2-AG) (Hillard, 2015). Although cannabinoid binding sites exist throughout the central nervous systems (CNS) and the peripheral nervous systems, circulating eCB concentrations are hypothesized to be biomarkers of CNS eCB signalling, and are also implicated in psychological depression (Hillard, 2018). Women with major depressive disorder (MDD) had lower serum 2-AG concentrations compared to non-depressed controls (Hill *et al.* 2009), and symptoms of depressed mood were inversely correlated with the 2-AG concentration (Stensson *et al.* 2018). Meanwhile, another study found significantly higher circulating concentrations of both AEA and 2-AG in MDD patients (Romero-Sanchiz *et al.* 2019). A study in a sizable group of participants with and without a history of psychiatric disorders showed a relationship between the circulating levels of eCBs and affect regulation (Coccaro *et al.* 2018). The strongest relationship was observed between the affect intensity and the circulating AEA levels, even in the absence of relationships categorised by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM)-5 or state dimensional measures of depression or anxiety (Coccaro *et al.* 2018). Furthermore, the eCBs system can play a role in the remission of depression. Administration of an eCB agonist to healthy volunteers induced a network-wide shift of the brain from a bias for negative emotional content towards a bias for positive emotional content (Bossong *et al.* 2013). The activation of the CB1R, which is encoded by the CB1R gene (*CNR1*), may be effective as a treatment for depression. By analysing the single nucleotide polymorphism (SNP) variability in the *CNR1* gene (rs806377) (Matsunaga *et al.* 2014), we showed that the subjective happiness level was higher in the C carriers, which have a high binding capability to the eCB receptor compared to the TT genotype. However, other researchers indicated no influence of the rs806377 on the overall susceptibility to MDD (Chakrabarti and Baron-Cohen, 2011).

AEA and 2-AG are the most well-studied eCBs, which are derived mainly from arachidonic acid (ARA), one of ω-6 polyunsaturated long-chain fatty acids (PUFAs) (Dyall, 2017, Hansen and Artmann, 2008). Moreover, AEA and 2-AG are degraded into ARA primarily by the serine hydrolase enzymes fatty acid amide hydrolase (FAAH) and mono-acylglycerol lipase (MAGL), respectively (Blankman & Cravatt, 2013). It was reported that depressive symptoms of white-collar workers were negatively correlated with the percentage of ARA in the serum (Tsuboi *et al.* 2013). Among the PUFAs, not only ARA but also ω-3 PUFAs are also involved in the function of the eCB system. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are major ω-3 PUFAs, which can convert to their ethanolamide derivatives docosahexaenoyl ethanolamine (DHEA) and eicosapentaenoyl ethanol-amide (EPEA), respectively. DHEA and EPEA exhibit binding affinity and agonist activity, though low, on eCB receptors (McDougle *et al.* 2017). Namely, DHEA and EPEA function as CB1R and CB2R agonists (Brown *et al.* 2010). Furthermore, cytochrome P450 epoxygenase (CYP) converts DHEA and EPEA into epoxydocosapentaenoic acid-ethanolamide (EDP-EA) and epoxides epoxyeicosatetraenoic acid-ethanolamide (EEQ-EA), respectively, both of which are CB1R and CB2R agonists (McDougle *et al.* 2017). In epidemiological studies, it is well-known that the intake of DHA or EPA has an inverse association with depression, especially in women (Colangelo *et al.* 2009, Tsuboi *et al.* 2019). Thus, DHA and EPA intake can be thought to be highly relevant with AEA and 2-AG functions.

We have been investigating factors that maintain psychological wellbeing in everyday life. Although MDD and depressive symptoms in the non-clinical population should be distinguished, assessing depressive symptoms and the prevention of depression is crucial, because depression is associated with an increased risk of mortality in general community populations, as well as in patient populations with chronic illnesses (Cuijpers *et al.* 2014). Additionally, in the last few years in Japan, coronavirus disease 2019 (COVID-19) pandemic increased the rates of probable depression by 2- to 9-fold during the second wave compared

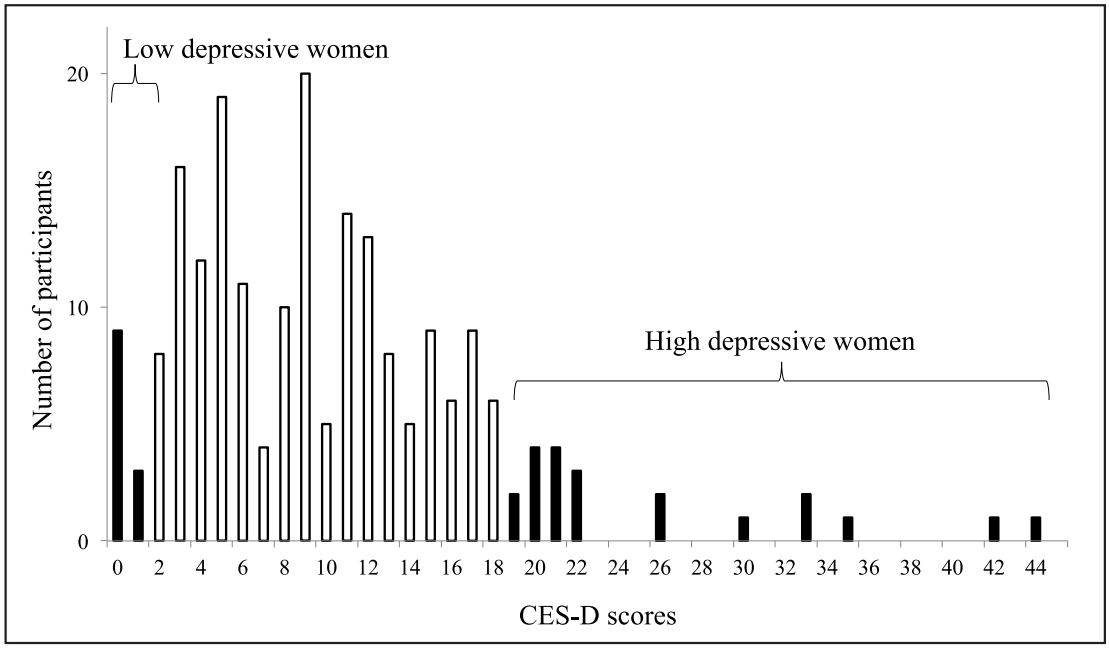

Fig. 1. Distribution of the Centre for Epidemiologic Studies Depression Scale (CES-D) scores among 208 female participants. Fourteen high depressive and ten low depressive women were selected for the present study.

with the pre-COVID-19 levels (Fukase *et al.* 2021). The COVID-19 pandemic is also contributed to the psychological status of many individuals all over the world as reviewed in previous articles. (Dong *et al.* 2021, Rodríguez-Fernández *et al.* 2021)

Thus, we hypothesized (1) that the serum eCB concentrations in low depressive people were higher compared with those in high depressive people, and (2) that people with low depressive symptoms tend to consume higher ARA, DHA, and EPA. In the present study, we selected high and low depressive women from community-dwelling people, and measured the serum concentrations of AEA and 2-AG. We also assessed the nutritional intake of PUFAs in the study participants. In addition, the rs806377 SNP of *CNR1* was considered as one of the covariates. The purpose of the present study was (1) to compare the AEA and 2-AG levels in the peripheral blood between the high and low depressive subjects, and (2) to investigate the associations among eCBs, nutrient intake, and de-pressive symptoms.

PARTICIPANTS AND METHODS

Study Population

The participants were selected from individuals in the "Shika study" project, which was conducted since 2011 in Noto Peninsula, Ishikawa, Japan. The details of this study have been reported elsewhere (Center, 2016). The study protocol was approved by the Ethical Committee at Kanazawa University on 18 December 2013 (receipt number 1491). Among 492 subjects aged 40 or older, 409 individuals [200 men: mean age 59.1 (standard deviation: S.D. 11.5), 208 women: mean age 61.1 (S.D. 12.9), 1 unknown] provided written informed consent on research participation. All the respondents were literate, understood the Japanese language well, and were requested not to use proxy respondents.

For the current study, we selected 14 high and 10 low depressive women that were age-matched as possible, using the Japanese version of the Centre for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977, Shima *et al.* 1985). The reason that we selected only women was because the association between depression and ω-3 PUFAs were stronger in women than in men (Colangelo *et al.* 2009), and because of sex matching.

Procedures and questionnaires

A self-administrated questionnaires were sent by post to the participants beforehand and collected on the examination day, when unclear parts of the questionnaires were addressed and corrected by the interviewers. The questionnaire contained demographic measures (age, sex, height, weight, present health status, diseases, medication, etc.), lifestyle characteristics (smoking status, alcohol consumption, etc.), and socio-economic status (living with family or alone, years of education, etc.). The entire process was conducted in a manner that protected the attendees' privacy.

Nutrient intake was assessed using the brief-type self-administered diet history questionnaire (BDHQ). The BDHQ is a four-page structured questionnaire that assesses the consumption frequency of 58 foods and beverages commonly consumed by the general Japanese population (Sasaki, 2004). It is a modified form of the self-administered diet history questionnaire (DHQ) (Sasaki *et al.* 1998), and was designed to be completed within approximately 15 minutes. The BDHQ estimates

Tab. 1. Comparison of variables between high and low depressive participants

Variable	High depressive (n=14)	Low depressive (n=10)	*p* value
Basic values			
Age	61.8 (13.56)	60.6 (12.8)	0.83
BMI	22.4 (3.49)	23.0 (3.67)	0.71
Depressive symptoms			
CES-D scores	24.4 (8.1)	0.8 (1.03)	<0.0005
SNP (rs806377)			
T/T	4	4	0.41
T/C	4	4	
C/C	2	0	
Smoking habit			
Non-smoker	12	9	0.24
Ex-smoker	0	1	
Present smoker	2	0	
Alcohol consumption			
No habit	11	8	0.93
Several days per week	3	0	
Everyday	0	2	
Years of education	11.5 (2.92)	10.4 (1.51)	0.31
Solitary life	2	1	0.37

Values are expressed as mean (S.D.), *p* values generated *t* test.

dietary intake in the last month using an ad hoc computer algorithm. The validity of the BDHQ has been demonstrated in previous studies (Kobayashi *et al.* 2011). In order to analyse nutrient data, the density method was used to estimate intake per 1000 kcal.

Blood Collection

Fasting blood was sampled between 08.00 a.m. and 12.00 p.m. from the forearm vein of each participant. The samples were collected into heparinized and se-rum-separator vacutainer tubes, from which sera and blood clots were obtained by incubating the tubes until the sera and clots were separated. The samples were then de-livered to Kanazawa University through a commercial laboratory (SRL Kanazawa Laboratory, Kanazawa, Japan) where the sera were dispensed into other tubes. The sera and the clots were frozen and stored at -30°C until the assay.

Measurement of AEA and 2-AG

The serum samples were analysed for AEA and 2-AG, using an ELISA kit for AEA (Cloud-Clone Corp., TX, USA) and an ELISA kit for 2-AG (Cloud-Clone Corp., TX, USA), respectively.

Genotyping of the CB1R Gene

Genomic DNA was extracted from blood lymphocytes using the QIAamp DNA Blood Maxi kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions or consigning the samples to a company specializing in clinical laboratory testing (SRL, Inc., Tokyo, Japan). Genome-wide SNP genotyping was performed using the Japonica Array v2 (Kawai *et al.* 2015) (TOSHIBA Co., Ltd. Tokyo, Japan). The quality control (QC) procedures for the obtained genome-wide SNP genotype data have been described previously (Nomura *et al.* 2021). Briefly, QC filtering of SNPs and participants was based on gender identity between karyotype and questionnaire, call rate, Hardy-Weinberg equilibrium test, inbreeding coefficient, cryptic relatedness, and population structure. The genotypes of SNP rs806377 (T/C), which is located in the *CNR1* gene, were extracted from the array data from 851 unrelated subjects (based on genome-wide π values), which passed the QC. In the QC step, the call rate for the SNP was 99.8%, and the deviation from the Hardy-Weinberg equilibrium was not observed ($p = .478$). Subsequently, 18 participants data were used in the present study.

Statistical Analysis

The Japanese version of the IBM SPSS Statistics version 25 software (IBM Japan, Tokyo, Japan) was utilized for data analysis. The high and low depressive individuals were compared using age, BMI, lifestyles, nutritional intake, and serum indicators. These comparisons were analysed with unpaired t-tests, followed by

a linear analysis to adjust for possible confounders. Subsequently, hierarchical models were applied to identify the effects of confounding factors. Chi-square test was applied for the comparison of the qualitative values. The results were considered to be statistically significant when $p < 0.05$.

RESULTS

As indicated in the method section, 14 participants with high depressive symptoms and 10 with low depressive symptoms were analysed (Figure 1). All the participants lived independently, and none used nursing services.

There were no significant differences in variables between the high and low depressive symptoms groups with the exception of CES-D scores (t-test: $p < 0.0005$) (Table 1); i.e., there were no significant differences in age, body mass index (BMI), *CNR1* SNP (rs806377), smoking habit, alcohol consumption, years of education, and whether the participant lives alone (Table 1: t-test for quantitative variables and Chi-square test for qualitative variables; $p = 0.83$, $p = 0.71$, $p = 0.41$, $p = 0.24$, $p = 0.93$, $p = 0.31$, $p = 0.35$, respectively). Meanwhile, t-test and linear models revealed that the ratio of the serum levels of AEA to ARA intake volume (AEA/ARA) in the high depressive group was significantly higher compared with those in the low depressive group (Model 1, 3, 4, 5: $p < 0.05$, $p < 0.05$, $p < 0.01$, $p < 0.01$, respectively) (Table 2). In contrast, there were no significant differences in the ratios of the serum 2-AG levels to PUFA intake volume (2-AG/ARA) as well as mere PUFA intake levels or serum eCBs levels between the high and low depressive groups (Table 2). The AEA/ARA data remained significant even after controlling for possible confounders including age, BMI, current smoking status, alcohol consumption, the *CNR1* SNP (rs806377), and whether the participant lives alone (Table 2). The eCBs/ARA distributions with the average scores and standard error (S.E.) were represented in Figure 2.

DISCUSSION

The present study compared the serum eCB (AEA and 2-AG) levels and daily intake volumes of PUFAs (ARA, DHA, and EPA) among high and low depressive female community-dwellers with possible confounders. We found a significant elevated level of AEA/ARA among high depressive participants, even when controlling for the confounders. Contrarily, there were no significant differences in other eCBs, PUFAs, or the ratios of eCBs/PUFAs between the high and low depressive participants. The reason that we evaluated the ratio of eCBs (AEA, 2-AG) to PUFAs (ARA, DHA, EPA) was that AEA and 2-AG derive from ARA, and that DHA and EPA (Dyall, 2017, Hansen & Artmann, 2008), including their metabolites DHEA, EPEA, EDP-EA and EEQ-EA, can work as CB1R and CB2R agonists (Brown *et al.* 2010).

This study did not confirm our hypothesis that low depressive individuals had higher serum levels of eCBs and higher intake of ARA, DHA, and EPA. Nonetheless, we identified a new finding. Specifically, the association between depressive degrees and AEA/ARA ratios in community-dwelling women.

Tab. 2. Comparison of variables between high and low depressive groups

Variables	High depressive (n=14)	Low depressive (n=10)	Model 1	Model 2	Model 3	Model 4	Model 5
			|*t*| value	Standardised |*β*| value			
Serum eCB concentrations							
AEA (ng/mL)	39.6 (29.25)	26.0 (23.73)	1.21	0.28	0.43	0.64	0.67
2-AG (ng/mL)	41.5 (33.91) [a]	34.3 (33.47)	0.50	0.19	0.32	0.56	0.53
Nutrients intake per day							
ARA (g/1000kcal)	78.3 (19.12)	100.6 (41.51)	1.58	0.04	0.09	0.19	
DHA (g/1000kcal)	262.6 (97.92)	378.4 (339.56)	1.05	0.14	0.04	0.05	
EPA (g/1000kcal)	157.9 (66.22)	231.2 (226.89)	0.99	0.15	0.05	0.02	
Ratio of eCB to ARA							
AEA/ARA	0.57 (0.480)	0.26 (0.183)	2.20*	0.43	0.59*	0.95**	0.96**
2-AG/ARA	0.53 (0.455) [a]	0.35 (0.301)	1.09	0.33	0.45	0.84	0.83

*: $p < 0.05$, **: $p < 0.01$, data are represented as the mean (S.D.: standard deviation)
Model 1: Simple comparison by unpaired t-test.
Model 2: Comparison adjusted for age and BMI by analysis of covariance.
Model 3: Model 2 + adjusted for current smoking and alcohol consumption by analysis of covariance.
Model 4: Model 3 + adjusted for living alone.
Model 5: Model 4 + adjusted for CNR1.
[a] n=12 due to small volume of two serum samples.
eCB: endocannabinoid, AEA: anandamide, 2-AG: 2-arachidonoylglycerol, PUFA: polyunsaturated fatty acids, ARA: arachidonic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid

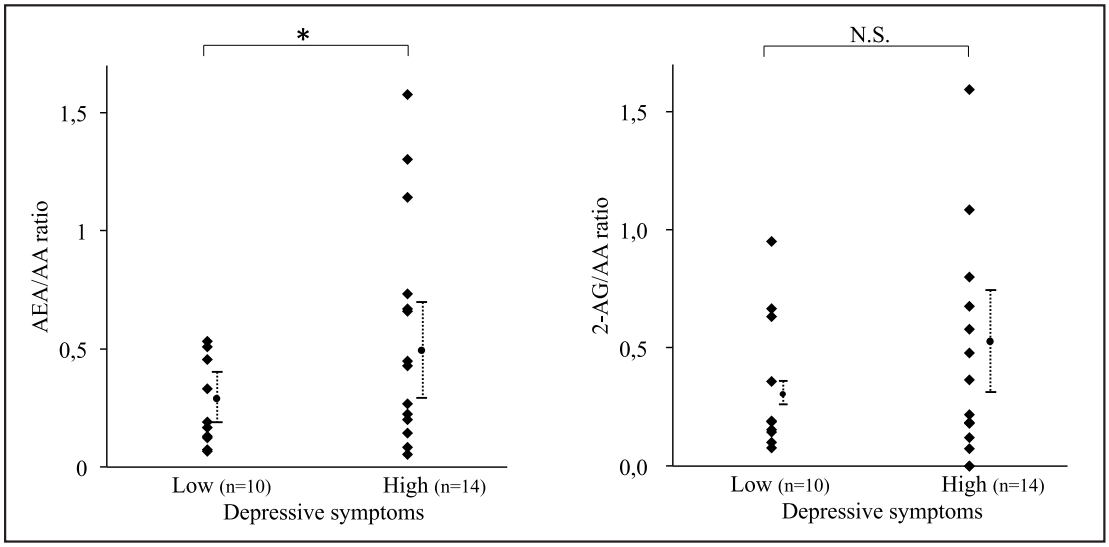

Fig. 2. Comparison of the ratio of the eCB serum level (ng/mL) to the PUFA intake volume (g/1000kcal) between high (n=14) and low (n=10) depressive participants. Dotted line represents average score and S.E. of each variable. The AEA/ARA ratio was significantly higher in high depressive participants than that of low depressive participants (t-test: $t = 2.22$, $p < 0.05$). The significance remained even after controlling for possible confounders including age, BMI, current smoking status, alcohol consumption, the *CNR1* SNP (rs806377), and whether the participants live alone (See Table 2). The depressive symptoms were assessed using CES-D.
*: $p < 0.05$, N.S.: not significant, eCB: endcannabinoid, PUFA: polyunsaturated long-chain fatty acid, CES-D: the Japanese version of the Centre for Epidemiologic Studies Depression Scale, S.E.: standard error

eCBs

The eCBs system influences a wide variety of neurobiological processes including affect regulation and emotionality as well as other neuropsychiatric functions (Coccaro *et al.* 2018). We have been focusing on the associations between eCBs and psychological depression with relevance to PUFAs. In the current study, the ratio of AEA/ARA in the high depressive group was significantly higher than those in the low depressive group. In addition, there was no significant difference in the ratio of 2-AG/ARA between the two groups. We analysed the ratios, because ARA is a precursor of AEA and 2-AG (Freitas *et al.* 2018, Hansen & Artmann, 2008). The AEA/ARA ratio was higher in the high depressive groups, which appears to contradict the hypothesis that eCB deficiency contributes to depressive symptoms. Below, we attempt to explain these contradictory results.

Elevated ratios of AEA/ARA in high depressive participants

Several studies have found that circulating concentrations of both AEA and 2-AG are significantly altered in women diagnosed with depression. For example, the serum concentrations of AEA and 2-AG in women with MDD were significantly lower than in matched controls (Hill *et al.* 2009). Another study reported a lower concentration of 2-AG in women newly diagnosed with MDD compared to controls. This study also revealed a significant negative relationship between circulating 2-AG and the duration of depressive symptoms (Hill *et al.* 2008). Contrarily, AEA concentrations were increased in women diagnosed with minor depression, which the authors argued could be a compensatory mechanism to maintain an elevated mood (Hill *et al.* 2009). A recent study of men and women indicated a significant inverse correlation between serum 2-AG levels and the severity of depressive symptoms of MDD patients assessed by the Beck Depression Inventory (BDI). Moreover, baseline plasma levels of AEA and 2-AG did not differ between MDD patients and healthy controls (Bersani *et al.* 2021). It remains an unanswered question whether circulating eCB concentrations are related to specific depression symptoms or clusters of symptoms.

In the present study, there was no significant differences in serum AEA and 2-AG levels between the high and low depressive groups. One explanation for this finding may be that the sample size was too small to detect any differences. Additionally, the CES-D scores of the high depressive group ranged from 17 to 33, with the exception of one MDD patient with a CES-D score 44. These CES-D scores may not be high enough to represent a high depressive group.

Functional Differences of AEA and 2-AG

Interestingly, only AEA/ARA, but not 2-AG/ARA, showed significant differences between the high and low depressive participants. These results may be due to the functional differences between AEA and 2-AG as well as different distributions of CB1R and CB2R throughout the body. 2-AG activates both CB1R and CB2R with similar potency, whereas AEA activates CB1R with a much higher potency than CB2R (McDougle *et al.* 2017). Additionally, CB1R is found

predominantly in the CNS, and CB2R is found in both the CNS and the peripheral immune cells (Ashton & Glass, 2007). Therefore, the function and distribution of AEA may elevate the AEA/ARA ratios in high depressive participants in order to compensate for maintaining psychological health.

Unique function of ARA, DHA, and EPA

ARA, DHA and EPA are essential fatty acids that humans cannot synthesize, so they need to be ingested for maintaining good health (Freitas *et al.* 2018). ARA is one of the main ω-6 PUFAs, which is converted into AEA and 2-AG (Dyall, 2017, Hansen & Artmann, 2008). On the other hand, DHA and EPA are major ω3 PUFAs, which are converted into DHEA and EPEA. CYP converts DHEA and EPEA into the eCB epoxides EDP-EA and EEQ-EA, respectively, both of which are CB1R and CB2R agonists (Brown *et al.* 2010, McDougle *et al.* 2017).

In the present study, there were no significant differences between high and low depressive groups in ARA, DHA, EPA, 2-AG/ARA, AEA/DHA, 2-AG/DHA, AEA/EPA, or 2-AG/EPA between the high and low depressive groups with the exception of AEA/ARA (Table 2). Given that DHA and EPA functions as agonists of CB1R and CB2R, the AEA/DHA, 2-AG/DHA, AEA/EPA, or 2-AG/EPA ratios in the high depressive group might be elevated to maintain the mental wellness. Since DHA and EPA reduced depressive symptoms, the intake volume of DHA and EPA in the high depressive group might be lower. However, the sample size of this study might be too small to establish significant results.

The presence or absence in the diet of certain PUFAs is associated with changes in AEA or 2-AG levels in several brain regions as well as various tissues (Matias *et al.* 2008). Although it is not possible to explain the results of the present study, the following reports may be pertinent to the results. For example, piglets with diets rich in ARA and poor in DHA were shown to have significantly enhanced levels of AEA in the brain (Berger *et al.* 2001). Moreover, in a rat study, ω-3 PUFA deficiency resulted in increased 2-AG in the whole brain and plasma of adults and developing animals (Watanabe *et al.* 2003), while supplementation of ω-3 PUFA appeared to reduce the AEA levels (Wood *et al.* 2010). In addition, supplementation of ω-3 PUFAs reduced 2-AG levels, which was associated with an increase in DHA incorporation within brain phospholipid membranes (Piscitelli *et al.* 2011).

Other variables

Although lifestyle (smoking habit and alcohol consumption), socio-economic status (years of education) and *CNR1* SNP (rs806377) might be related to depressive symptoms, there were no significant differences in these variables between the high and low depressive participants in the current study. However, these variables may be meaningful as confounding factors.

Limitations

Firstly, the serum levels of PUFAs were not measured. Although we monitored ARA intake, the analysis of serum ARA concentrations to analysis would be useful to elucidate the mechanism of ARA metabolism, because AEA and 2-AG are derived mainly from ARA (Dyall, 2017, Hansen & Artmann, 2008), and are degraded into ARA again (Blankman & Cravatt, 2013). Secondly, since this was a cross-sectional study, causal associations cannot be inferred. Thirdly, the number of participants was small. We had selected 18 high and 18 low depressive women that were age-matched as possible. This sample size was chosen because the eCB ELISA kit for could accommodate 36 samples. However, we mistakenly measured 12 serum samples collected two years earlier (2013) with no CES-D results, because some of the numbers written on the sample tubes were the same between 2015 and 2013. Consequently, 14 serum samples from high depressive women and 10 serum samples from low depressive women were used in the present analysis. Finally, also other variables may be significantly different in women with high depressive disorder in larger sample.

Conclusions

In summary, among female community-dwellers of Japan, we found an elevated ratio of serum AEA to daily intake of ARA in high depressive participants compared with those in low depressive participants. This result suggested that the conversion rate of ARA to AEA may be accelerated in depressive individuals. ARA supplementation may be helpful to maintain psychological wellbeing in daily life, although further studies are needed to address this hypothesis.

ACKNOWLEDGEMENTS

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DISCLOSURE STATEMENT

Nothing to disclose.

FUNDING

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

H.T. (Hirohito Tsuboi) performed the psychological assessment of the study, the data analyses, and drafted the manuscript. M.M. contributed to the AEA and 2-AG detection. H.T. (Hiromasa Tsujiguchi) and A.H. managed the participation of the subjects. T.K., A.T., K.H., and T.S. detected the SNPs. N.Y. curated data. H.N. conducted the whole cohort study.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethical Committee at Kanazawa University (protocol code 1491 on 18 December 2013).

INFORMED CONSENT STATEMENT

Written informed consent has also been obtained from the participants to publish this paper.

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