ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Nutrition in Health and Disease

Nonesterified Fatty Acids and Depression in Cancer Patients and Caregivers

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ABSTRACT

Background: Nonesterified fatty acids (NEFAs) are known to have inflammatory effects. The inflammatory hypothesis of depression suggests that omega-3 (ω-3) and omega-6 (ω-6) fatty acids might be negatively and positively correlated with depression, respectively.

Objective: An exploratory study was conducted to determine the association between dietary free fatty acids and depressive symptoms in cancer patients and caregivers.

Methods: Associations between depression and the NEFA pool were investigated in 56 cancer patients and 23 caregivers using a combination of nonparametric tests and regularized regression. Plasma NEFAs were measured using thin layer and gas chromatography with flame ionization detection. Depression was characterized both as a continuous severity score using the GRID-Hamilton Depression Rating Scale (GRID Ham-D), and as a categorical diagnosis of major depression by structured clinical interview.

Results: Initial hypotheses regarding the relation between depression and omega-3 or omega-6 fatty acids were not well supported. However, elaidic acid, a *trans*-unsaturated fatty acid found in hydrogenated vegetable oils, was found to be negatively correlated with continuous depression scores in cancer patients. No significant associations were found in caregivers.

Conclusions: An unexpected negative association between elaidic acid and depression was identified, supporting recent literature on the role of G protein–coupled receptors in depression. Further research is needed to confirm this result and to evaluate the potential role of G protein agonists as therapeutic agents for depression in cancer patients. *Curr Dev Nutr* 2020;4:nzaa156.

Keywords: cytokines, IL-1ra, nonesterified fatty acid, elaidic acid, GPR40, GPR120, machine learning, LASSO

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Abbreviations used: ALA, α-linolenic acid; CIRS, compensatory anti-inflammatory reflex system; DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders, fourth edition*; FDR, false discovery rate; GPR40, G protein–coupled receptor 40; GRID Ham-D, GRID-Hamilton Depression Rating Scale; LASSO, least absolute shrinkage and selection operator; NEFA, nonesterified fatty acid; SCID, Structured Clinical Interview for DSM-IV; Th, T-helper; TLE, total lipids extract.

Introduction

Dietary PUFAs are critical for normal neuronal development and function and are hypothesized to influence psychiatric disorders, including depression (1). Despite strong interest in identifying fatty acids that could behave as therapeutic agents for depression, dietary studies have yielded relatively weak results (2), although there are some meta-analyses showing early promise of omega-3 (ω-3) fatty acids in the treatment of depression (3, 4). It has become increasingly clear that the primary source of fatty acids in the brain is the nonesterified fatty acid (NEFA) pool, which readily crosses the blood–brain barrier (5). Therefore, investigating the NEFA pool in relation to depression presents a promising avenue for exploration.

One of the emerging theories for the pathophysiology of depression is the inflammatory hypothesis of depression (6, 7) and the related compensatory anti-inflammatory reflex system (CIRS) (8). Substantial evidence over the past decade supports the role of proinflammatory cytokines inducing microglial overactivation, increased indoleamine 2,3-dioxygenase activity, hypothalamic-pituitary-adrenal (HPA) axis dysregulation, and production of neurotoxic reactive oxygen species leading to depression (9). Many studies have shown an association between proinflammatory

markers such as IL-6 and TNF-α with depression (10). Targeted anti-inflammatory therapies are a promising new direction for treating depression.

Some NEFAs (particularly ω-6 and ω-3 fatty acids) are also known to have pro- and anti-inflammatory effects, respectively, and may play a role in depression indirectly through effects on inflammation (11). Several studies have identified associations between fatty acids and cytokines (12, 13), suggesting that the influence of NEFAs on depression may be mediated by cytokines.

The inflammatory hypothesis of depression has considerable potential for translational medicine related to cancer patients (10). Cancer patients experience high levels of inflammation resulting from tumor cell burden, treatment-induced tissue destruction, and psychological stress, as well as high rates of depression (14). Currently available antidepressants are less effective in cancer patients, partly due to the persisting influence of physical symptoms from medical illness (15). Caregivers also suffer from high rates of depression (16, 17), subject to psychological stress but not the biological stresses of cancer. Previous work aimed at addressing potential connections between inflammation and depression suggests that underlying biological mechanisms may differ between these groups (18). A role for NEFAs in the treatment of depressive symptoms in cancer patients and/or caregivers would be particularly valuable as dietary interventions are more palatable than medical interventions.

An exploratory study was conducted to assess the relation between the plasma NEFA pool and depression in cancer patients and caregivers. Specific hypotheses were that anti-inflammatory ω-3 NEFAs would be negatively associated with depression and that proinflammatory ω-6 NEFAs would be positively associated with depression. Since our methodology also captured other fatty acids, we included examinations of saturated and both *cis* and *trans* MUFAs. An analysis of *trans* fatty acids is particularly of interest as intakes have been correlated with depression risk and symptoms (19, 20). Furthermore, despite voluntary efforts to remove industrially produced *trans* fat–containing foods in Canada (21), young adults appear to have increased intakes over time (22).

Methods

This is a secondary analysis of a cross-sectional study population originally recruited to examine the relation between cytokines and depression in cancer, where concentrations of inflammatory cytokines were found to increase from healthy controls, to healthy caregivers, to cancer patients, associated with depressive symptoms (23). Based on meta-analytic effect sizes for the association between depression and fatty acids of −0.33 and −0.85 for ω-6 and ω-3 fatty acids, respectively (24), the fixed sample size of the original study would have the power to detect effect sizes of 0.3 for ω-6 fatty acids and 0.9 for ω-3 fatty acids (1-tailed, Wilcoxon test, $\alpha = 0.05$). Concentrations of NEFAs were measured from frozen plasma and associated with depression levels and cytokine concentrations in cancer patients and their primary caregivers. The STROBE cross-sectional reporting guidelines were used (25).

Subject recruitment

Cancer patients and their healthy primary caregivers were recruited from the outpatient oncology clinics at the Princess Margaret Cancer Centre in Toronto, Canada. Recruitment initially focused on stage III or IV gastrointestinal and lung cancer patients and their healthy caregivers. To increase recruitment, patients with any cancer type referred to the Psychosocial Oncology Clinic and their caregivers were also approached. Primary caregivers (either a spouse or partner of the patient, a relative or other family member, or a close friend) were identified by patients who had consented to participate in this study. Healthy individuals, recruited through advertisements in the surrounding university and hospital networks, were included as a comparison group. In total, 56 cancer patients, 23 primary caregivers, and 33 healthy controls were included in this study. Six of the cancer patients were re-assessed at a second time point as a follow-up.

Exclusion criteria intended to minimize variability in cytokine concentrations and symptom presentation included brain malignancy or history of neurological illness or trauma, substance abuse or dependence within 1 y, immunization within 30 d, blood donation within 60 d, pregnancy or use of hormonal contraceptives within 3 mo, and the presence of any psychiatric comorbidity (except depression) identified by the Mini International Neuropsychiatric Interview. Healthy controls were excluded from participating in the study if they were diagnosed with major depression based on the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders, fourth edition* (DSM-IV) (SCID) (26).

All study subjects provided informed consent for participation and this study received Research Ethics Board approval from the University Health Network, Toronto. Approved protocols were followed. All study participants were assigned a unique participant identification number, and after collection, all study records and data were stored by this number on encrypted, password-protected computers. Raw data were only accessible to authorized members of the research team.

Data collection

Depression data and covariates.

Depression was quantified as both a categorical variable [diagnosis of major depression by the SCID (26)] and as a continuous score on a depression severity rating scale, the 17-item GRID-Hamilton Depression Rating Scale (GRID Ham-D) (27), administered by a single trained research assistant just prior to blood collection.

Medical and demographic variables for cancer patients were extracted from the electronic medical record. Health behaviors and medical history were collected on a study questionnaire to calculate the Charlson comorbidity index and to capture frequency (or history) of smoking, alcohol use (type, amount, and frequency), caffeine consumption (type, amount, and frequency), as well as exercise habits (1, sedentary; 2, mild regular exercise; 3, occasional vigorous exercise; or 4 regular vigorous exercise).

NEFA and cytokine concentrations.

To minimize variability, plasma for all subjects was drawn between 08:00 and 10:00 h following an overnight fast of at least 12 h; premenopausal women were to be in the follicular phase of the menstrual cycle (confirmed by plasma estradiol and progesterone concentrations); no strenuous physical exercise and no alcohol consumption was to oc-

TABLE 1 Subject characteristics of cancer patients and caregivers with and without major depression and healthy controls[1]

	Cancer patients with MD ($n = 19$)	Cancer patients without MD ($n = 37$)	Caregivers with MD ($n = 3$)	Caregivers without MD ($n = 20$)	Healthy controls ($n = 33$)	*P*
Demographics						
Gender (female), *n* (%)	13 (68.4)	18 (48.6)	3 (100.0)	12 (60.0)	17 (51.5)	0.62
	31 (55.4)		15 (65.2)			
Mean income (SD)	$93K ($51K)	$88K ($39K)	$215K ($66K)	$91K ($30K)	–	0.13
	$90K ($43K)		$104K ($50K)			
Mean age (SD), y	50.7 (8.1)	58.0 (13.0)	46.7 (12.7)	58.2 (11.9)	44.8 (11.8)	<0.001
	55.5 (12.0)		56.6 (12.4)			
Ethnicity, *n* (%)						
Caucasian	13 (68.4)	33 (89.2)	2 (66.7)	18 (90.0)	23 (69.7)	0.21
	46 (82.1)		20 (87.0)			
Asian	1 (5.3)	3 (8.1)	1 (33.3)	2 (10.0)	6 (18.2)	
	4 (7.1)		3 (13.0)			
Other	5 (26.3)	1 (2.7)	0	0	4 (12.1)	
	6 (10.7)		0			
Biobehavioral variables						
GRID Ham-D (SD)	16.6 (7.1)	7.7 (5.4)	22.7 (5.5)	5.3 (4.8)	1.7 (2.1)	<0.001
	10.8 (7.3)		7.6 (7.6)			
Antidepressant use, *n* (%)	3 (15.7)	4 (10.8)	0	1 (5.0)	0	0.07
	7 (12.5)		1 (4.3)			
Mean BMI (SD), kg/m^2	26.6 (6.8)	24.5 (5.0)	–	–	–	
Smokers, *n* (%)	2 (10.5)	5 (13.5)	0.0	3 (15.0)	6 (18.2)	0.77
	7 (12.5)		3 (13.0)			
Mean alcohol consumption (SD), drinks/wk	1.2 (2.8)	3.8 (6.5)	3.7 (3.5)	2.3 (3.0)	2.0 (2.7)	0.68
	2.9 (5.7)		2.5 (3.1)			
Mean caffeine (SD), drinks/wk	18.0 (12.4)	20.7 (20.1)	18.7 (14.6)	21.6 (14.5)	14.4 (11.4)	0.15
	19.8 (17.8)		21.2 (14.2)			
Mean exercise habits (SD)[2]	1.6 (0.8)	1.8 (0.7)	2.7 (1.2)	1.9 (0.7)	2.4 (0.8)	<0.001
	1.7 (0.7)		2.0 (0.8)			
Cancer characteristics, *n* (%)						
GI cancer	7 (36.8)	14 (37.8)				
Lung cancer	0 (0.0)	9 (24.3)				
Breast cancer	3 (15.7)	2 (5.4)				
Gynecological cancer	4 (21.1)	2 (5.4)				
Hematological cancer	3 (15.8)	6 (16.2)				
Skin cancer	1 (5.3)	1 (2.7)				
Unknown primary cancer	1 (5.3)	3 (8.1)				
Cancer stage						
Early stage (1/2)	5 (26.3)	5 (13.5)				
Late stage (3/4)	10 (52.6)	25 (67.6)				
Hematologic	4 (21.1)	7 (18.9)				
Treatment status						
No active treatment	12 (63.2)	16 (43.2)				
Active treatment	7 (36.8)	21 (56.8)				

[1]Mean values are provided and SDs are in parentheses unless otherwise stated. *P* values refer to whether subject characteristics differed among cancer patients, caregivers, and healthy controls irrespective of depression status. GI, gastrointestinal; GRID Ham-D, GRID-Hamilton Depression Rating Scale; MD, major depression.
[2]Exercise habits were rated as 1 – sedentary, 2 – mild regular, 3 – occasional vigorous, 4 – regular vigorous.

cur for 48 h prior to blood draw. The sample collection was postponed if participants had an acute or infectious illness, allergic reaction, physical injury, or dental work in the 2 wk prior to the scheduled sampling visit. Blood samples were collected in EDTA Vacutainer® tubes (BD, Franklin Lakes, NJ), with plasma separated within 2 h of collection and frozen in aliquots at −70°C until assayed.

Multiplexed electrochemiluminescence cytokine immunoassays were performed on singly thawed aliquots using the Meso Scale Discovery (Meso Scale Diagnostics, Rockville, MD) system [as previously described (23)], consisting of a panel of 15 T-helper (Th) 1/Th2 cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, TNF-α, IL-6, IL-1ra, IL-12p40, IL-17, and IL-2R). All assays were conducted in duplicate wells with mean values analyzed, from a single manufacturer lot number. Samples measuring below the lower limit of quantification for the assay were treated as zero.

For NEFA assays, plasma total lipids extracts (TLEs) were isolated in the presence of a known amount of heptadecanoic acid (17:0) as an internal standard. To isolate NEFAs, the TLEs were loaded onto prewashed TLC plates (TLC silica gel 60; EMD Millipore) and run in heptane:diethyl ether:glacial acetic acid (60:40:2, vol:vol:vol), identified under UV light, collected and transferred to a glass test tube, as described previously (28). NEFAs were transmethylated using 14% boron

TABLE 2 Kruskal-Wallis tests comparing FFAs in cancer patients, caregivers, and healthy controls[1]

		Weight %			Concentration		
FFA	Type	Kruskal-Wallis χ^2	*P*	FDR	Kruskal-Wallis χ^2	*P*	FDR
Caprylic acid, 8:0		0.35	0.84	0.92	0.23	0.89	0.98
Lauric acid, 12:0		0.28	0.87	0.92	0.04	0.98	0.98
Myristic acid, 14:0		0.27	0.87	0.92	0.26	0.88	0.98
Myristoleic acid, 14:1n-5		2.84	0.24	0.91	2.58	0.28	0.98
Pentadecylic acid, 15:0		0.25	0.88	0.92	0.65	0.72	0.98
Palmitic acid, 16:0		2.78	0.25	0.91	2.39	0.30	0.98
Palmitelaidic acid, *trans*-16:1n-7		0.50	0.78	0.92	1.18	0.56	0.98
Palmitoleic acid, 16:1n-7		7.88	0.02*	0.36	3.33	0.19	0.98
Sum of 16:1n-7		8.37	0.02*	0.36	3.20	0.20	0.98
Stearic acid, 18:0		3.51	0.17	0.91	0.13	0.94	0.98
Elaidic acid, *trans*-18:1n-9		0.17	0.92	0.92	0.86	0.65	0.98
Oleic acid, 18:1n-9		2.02	0.36	0.91	0.83	0.66	0.98
Sum of 18:1n-9		2.14	0.34	0.91	0.84	0.66	0.98
Vaccenic acid, 18:1n-7		3.77	0.15	0.91	1.35	0.51	0.98
Linoelaidic acid, *trans/trans*-18:2n-6	ω-6	1.71	0.43	0.91	1.66	0.44	0.98
Linoelaidic acid, *cis/trans*-18:2n-6	ω-6	1.26	0.53	0.92	0.86	0.65	0.98
Linoelaidic acid, *trans/cis*-18:2n-6	ω-6	1.35	0.51	0.92	0.09	0.96	0.98
Linoleic acid, 18:2n-6	ω-6	2.80	0.25	0.91	0.31	0.86	0.98
Sum of 18:2n-6	ω-6	2.66	0.26	0.91	0.29	0.86	0.98
Arachidic acid, 20:0		4.67	0.10	0.91	4.84	0.089	0.98
α-Linolenic acid, 18:3n-6	ω-6	0.21	0.90	0.92	0.83	0.66	0.98
Gondoic acid, 20:1n-9		1.57	0.46	0.91	0.57	0.75	0.98
α-Linolenic acid, 18:3n-3	ω-3	0.98	0.61	0.92	1.18	0.55	0.98
Conjugated linoelaidic acid, *trans/trans*-18:2n-6	ω-6	0.39	0.82	0.92	1.21	0.55	0.98
Eicosadienoic acid, 20:2n-6	ω-6	0.84	0.66	0.92	0.72	0.70	0.98
Behenic acid, 22:0		0.82	0.66	0.92	0.49	0.78	0.98
Dihomo-γ-linolenic acid, 20:3n-6	ω-6	0.21	0.90	0.92	0.90	0.64	0.98
Erucic acid, 22:1n-9		1.85	0.40	0.91	0.56	0.76	0.98
Eicosatrienoic acid, 20:3n-3	ω-3	2.31	0.31	0.91	2.41	0.30	0.98
Arachidonic acid, 20:4n-6	ω-6	1.52	0.47	0.91	0.57	0.75	0.98
Lignoceric acid, 24:0		0.66	0.72	0.92	0.20	0.91	0.98
EPA, 20:5n-3	ω-3	2.00	0.37	0.91	4.42	0.11	0.98
Nervonic acid, 24:1n-9		3.53	0.17	0.91	1.65	0.44	0.98
Adrenic acid, 22:4n-6	ω-6	1.53	0.47	0.91	0.46	0.79	0.98
n-6 DPA, 22:5n-6	ω-6	0.76	0.68	0.92	0.23	0.89	0.98
n-3 DPA, 22:5n-3	ω-3	0.75	0.69	0.92	0.21	0.90	0.98
DHA, 22:6n-3	ω-3	0.26	0.88	0.92	0.14	0.94	0.98

[1] FFAs were quantified as either weight % or concentration. DPA, docosapentaenoic acid; FDR, false discovery rate; FFA, free fatty acid. *indicates statistical significance.

trifluoride in methanol at 100°C for 1 h. FAMEs were isolated with hexane and quantified using GC–flame ionization detection. FAMEs were analyzed using a Varian-430 gas chromatograph (Varian, Lake Forest, CA) equipped with a Supelco SP-2560 fused silica capillary column (100-m × 0.25-mm interior diameter × 0.20-μm film thickness) and a flame ionization detector, as previously described. Peaks were identified by retention times of authentic FAME standards (Nu-Chek Prep). The concentration of each fatty acid was calculated by comparison with the heptadecanoic acid internal standard.

Data analysis

Statistical analysis.

NEFA concentrations were compared across the 3 sample groups (cancer patients, caregivers, and healthy controls) using the nonparametric Kruskal-Wallis test to determine if NEFA concentrations in cancer patients and caregivers were typical of the general population, and Wilcoxon rank-sum test for pairwise comparisons for categories with at least 3 patients. Associations between NEFA concentrations and total depression scores were evaluated with Spearman rank correlation analysis within cancer patients and within caregivers. Cancer patients with follow-up data were used to assess the potential prognostic value of NEFAs significantly associated with depression. All analyses were conducted using NEFA concentration data as well as with weight percentage of NEFAs. Nominal *P* values were corrected for multiple testing using the false discovery rate (FDR) method (29). Multivariate regression analysis was conducted to evaluate all NEFAs simultaneously and to include covariates. Age, gender, and exercise habits were included as covariates for both cancer patients and caregivers. For cancer patients, cancer type, cancer stage, and active treatment were included as unordered factors, and analyses were conducted with and without this set of covariates. The impact of BMI on NEFA concentrations was as-

TABLE 3 Wilcoxon test comparing SCID-categorized depressed cancer patients versus nondepressed cancer patients with FFAs quantified as weight percentage and concentration[1]

		Weight %			Concentration		
FFA	Type	W	*P*	FDR	W	*P*	FDR
Caprylic acid, 8:0		340	0.85	0.98	334	0.77	0.95
Lauric acid, 12:0		355	0.96	0.99	368	0.78	0.95
Myristic acid, 14:0		346	0.93	0.98	389	0.52	0.93
Myristoleic acid, 14:1n-5		317	0.56	0.90	328	0.69	0.95
Pentadecylic acid, 15:0		437	0.14	0.44	450	0.09	0.66
Palmitic acid, 16:0		437	0.14	0.44	404	0.37	0.93
Palmitelaidic acid, *trans*-16:1n-7		441	0.12	0.44	410	0.32	0.90
Palmitoleic acid, 16:1n-7		384	0.58	0.90	380	0.63	0.93
Sum of 16:1n-7		381	0.62	0.90	380	0.63	0.93
Stearic acid, 18:0		392	0.49	0.85	444	0.11	0.66
Elaidic acid, *trans*-18:1n-9		513	0.01*	0.17	473	0.04*	0.45
Oleic acid, 18:1n-9		305	0.43	0.79	347	0.94	0.99
Sum of 18:1n-9		325	0.66	0.90	347	0.94	0.99
Vaccenic acid, 18:1n-7		362	0.86	0.98	367	0.80	0.95
Linoelaidic acid, *trans/trans*-18:2n-6	ω-6	455	0.08	0.44	436	0.15	0.66
Linoelaidic acid, *cis/trans*-18:2n-6	ω-6	380	0.63	0.90	389	0.52	0.93
Linoelaidic acid, *trans/cis*-18:2n-6	ω-6	427	0.20	0.52	414	0.29	0.88
Linoleic acid, 18:2n-6	ω-6	240	0.05	0.40	311	0.49	0.93
Sum of 18:2n-6	ω-6	259	0.11	0.44	319	0.58	0.93
Arachidic acid, 20:0		344	0.90	0.98	353	0.99	0.99
α-Linolenic acid, 18:3n-6	ω-6	312	0.50	0.85	338	0.82	0.95
Gondoic acid, 20:1n-9		437	0.14	0.44	433	0.16	0.66
α-Linolenic acid, 18:3n-3	ω-3	227	0.03*	0.33	285	0.25	0.85
Conjugated linoelaidic acid, *trans/trans*-18:2n-6	ω-6	279	0.21	0.53	310	0.48	0.93
Eicosadienoic acid, 20:2n-6	ω-6	400	0.41	0.79	387	0.54	0.93
Behenic acid, 22:0		473	0.04*	0.33	494	0.01*	0.45
Dihomo-γ-linolenic acid, 20:3n-6	ω-6	353	0.99	0.99	369	0.77	0.95
Erucic acid, 22:1n-9		443	0.12	0.44	473	0.04*	0.45
Eicosatrienoic acid, 20:3n-3	ω-3	283	0.24	0.54	306	0.44	0.93
Arachidonic acid, 20:4n-6	ω-6	344	0.90	0.98	354	0.97	0.99
Lignoceric acid, 24:0		335	0.78	0.98	342	0.88	0.98
EPA, 20:5n-3	ω-3	271	0.17	0.48	283	0.24	0.85
Nervonic acid, 24:1n-9		305	0.43	0.79	321	0.60	0.93
Adrenic acid, 22:4n-6	ω-6	328	0.69	0.92	368	0.78	0.95
n-6 DPA, 22:5n-6	ω-6	419	0.25	0.54	435	0.15	0.66
n-3 DPA, 22:5n-3	ω-3	364	0.84	0.98	385	0.57	0.93
DHA, 22:6n-3	ω-3	245	0.07	0.44	265	0.13	0.66

[1]DPA, docosapentaenoic acid; FDR, false discovery rate; FFA, free fatty acid; SCID, Structured Clinical Interview for DSM-IV. *indicates statistical significance.

sessed in a subset of cancer patients for whom BMI data were available ($n = 49$).

Given the large number of variables relative to sample size, we used least absolute shrinkage and selection operator (LASSO) regularization to select the most important variables during linear regression. Although LASSO has been widely used for model selection, methods to evaluate the statistical significance of adaptive or "data-driven" approaches have only recently been developed. One such approach is the covariance test statistic (30), implemented with the covTest package in R (R Core Team). The covariance test statistic measures how much covariance between the outcome and the fitted model can be attributed to each new predictor. Furthermore, the covariance test statistic identifies appropriate *P* values by balancing the opposing forces of adaptivity, which increases variance and the potential for overfitting, and shrinkage, which reduces overfitting (30).

Regularized regression analysis was conducted for both cancer patients and caregivers using both NEFA concentrations and weight percentage. Regularized logistic regression analysis was conducted to evaluate associations with major depression in cancer patients. NEFAs and numeric covariates were $\log_2$ transformed and standardized prior to analysis. Categorical variables (cancer type, treatment, stage) were converted to binary dummy variables and standardized. NEFA values of zero (found in 2 samples for DHA) were converted to 0.001 to allow for log transformation. Total depression scores were square root transformed in caregivers so that residuals of regression analyses would be normally distributed. Sums of related NEFAs were removed from the datasets to reduce multicollinearity of explanatory variables. All statistical analysis was conducted in R.

NEFAs and cytokines.

A potential connection between NEFAs, cytokines, and depression was explored if any NEFAs were found to have significant associations with depression. Correlation analysis between NEFAs and cytokine concentrations was conducted to examine whether NEFAs might exert a direct

TABLE 4 Spearman rank correlation results of total depression scores with FFAs quantified as weight percentage and concentration for cancer patients and caregivers[1]

		Weight %						Concentration					
		Cancer patients			Caregivers			Cancer patients			Caregivers		
FFA	Type	ρ	*P*	FDR	ρ	*P*	FDR	ρ	*P*	FDR	ρ	*P*	FDR
Caprylic acid, 8:0		−0.11	0.41	0.83	0.10	0.66	0.91	0.07	0.63	0.80	0.07	0.76	0.91
Lauric acid, 12:0		0.04	0.74	0.91	0.30	0.17	0.62	0.12	0.38	0.56	0.24	0.27	0.91
Myristic acid, 14:0		−0.18	0.18	0.66	−0.10	0.66	0.91	−0.03	0.82	0.87	−0.11	0.61	0.91
Myristoleic acid, 14:1n-5		−0.10	0.46	0.83	0.06	0.79	0.92	0.01	0.96	0.96	−0.01	0.97	0.99
Pentadecylic acid, 15:0		−0.17	0.20	0.66	0.14	0.52	0.86	−0.05	0.69	0.81	0.07	0.77	0.91
Palmitic acid, 16:0		−0.03	0.83	0.91	−0.02	0.94	0.96	0.13	0.36	0.56	−0.29	0.18	0.91
Palmitelaidic acid, *trans*-16:1n-7		−0.32	0.02*	0.28	−0.17	0.44	0.86	−0.05	0.73	0.81	−0.14	0.53	0.91
Palmitoleic acid, 16:1n-7		0.15	0.28	0.66	−0.14	0.52	0.86	0.21	0.13	0.48	−0.20	0.37	0.91
Sum of 16:1n-7		0.14	0.29	0.66	−0.20	0.36	0.80	0.20	0.14	0.48	−0.18	0.41	0.91
Stearic acid, 18:0		−0.23	0.09	0.47	0.14	0.51	0.86	0.07	0.61	0.80	−0.07	0.76	0.91
Elaidic acid, *trans*-18:1n-9		−0.51	<0.01*	0.00*	0.01	0.96	0.96	−0.25	0.06	0.37	−0.06	0.79	0.91
Oleic acid, 18:1n-9		0.18	0.19	0.66	−0.31	0.15	0.62	0.22	0.11	0.48	−0.14	0.52	0.91
Sum of 18:1n-9		0.09	0.49	0.83	−0.33	0.13	0.62	0.20	0.13	0.48	−0.18	0.41	0.91
Vaccenic acid, 18:1n-7		0.18	0.19	0.66	−0.34	0.11	0.62	0.21	0.12	0.48	−0.14	0.53	0.91
Linoelaidic acid, *trans/trans*-18:2n-6	ω-6	−0.04	0.79	0.91	−0.33	0.13	0.62	0.12	0.37	0.56	−0.27	0.21	0.91
Linoelaidic acid, *cis/trans*-18:2n-6	ω-6	−0.05	0.70	0.91	0.14	0.53	0.86	0.13	0.32	0.56	0.05	0.82	0.91
Linoelaidic acid, *trans/cis*-18:2n-6	ω-6	−0.01	0.96	0.99	−0.09	0.70	0.91	0.12	0.37	0.56	−0.05	0.82	0.91
Linoleic acid, 18:2n-6	ω-6	0.27	0.05*	0.44	0.03	0.88	0.96	0.30	0.02*	0.31	−0.06	0.78	0.91
Sum of 18:2n-6	ω-6	0.24	0.08	0.47	0.08	0.71	0.91	0.28	0.03*	0.31	−0.05	0.83	0.91
Arachidic acid, 20:0		−0.15	0.28	0.66	−0.07	0.75	0.92	−0.02	0.86	0.89	0.00	0.99	0.99
α-Linolenic acid, 18:3n-6	ω-6	0.05	0.72	0.91	0.34	0.12	0.62	0.18	0.18	0.48	0.17	0.45	0.91
Gondoic acid, 20:1n-9		0.00	0.99	0.99	0.26	0.23	0.68	0.07	0.62	0.80	0.26	0.22	0.91
α-Linolenic acid, 18:3n-3	ω-3	0.30	0.02*	0.28	0.09	0.68	0.91	0.33	0.01*	0.31	0.02	0.92	0.97
Conjugated linoelaidic acid, *trans/trans*-18:2n-6	ω-6	0.07	0.62	0.91	0.25	0.26	0.68	0.27	0.05*	0.34	0.16	0.47	0.91
Eicosadienoic acid, 20:2n-6	ω-6	−0.07	0.62	0.91	0.03	0.90	0.96	0.11	0.42	0.60	0.06	0.79	0.91
Behenic acid, 22:0		−0.23	0.09	0.47	0.01	0.95	0.96	−0.13	0.35	0.56	0.05	0.81	0.91
Dihomo-γ-linolenic acid, 20:3n-6	ω-6	−0.04	0.79	0.91	0.32	0.14	0.62	0.18	0.18	0.48	0.23	0.30	0.91
Erucic acid, 22:1n-9		−0.16	0.24	0.66	0.14	0.51	0.86	−0.04	0.75	0.81	0.12	0.59	0.91
Eicosatrienoic acid, 20:3n-3	ω-3	0.14	0.29	0.66	−0.09	0.70	0.91	0.29	0.03*	0.31	−0.14	0.51	0.91
Arachidonic acid, 20:4n-6	ω-6	−0.03	0.81	0.91	0.34	0.11	0.62	0.19	0.17	0.48	0.21	0.35	0.91
Lignoceric acid, 24:0		−0.10	0.46	0.83	0.25	0.25	0.68	0.05	0.74	0.81	0.11	0.62	0.91
EPA, 20:5n-3	ω-3	0.04	0.77	0.91	0.31	0.15	0.62	0.17	0.22	0.50	0.31	0.14	0.91
Nervonic acid, 24:1n-9		0.03	0.81	0.91	0.48	0.02*	0.62	0.14	0.30	0.56	0.43	0.04*	0.91
Adrenic acid, 22:4n-6	ω-6	0.04	0.79	0.91	0.06	0.77	0.92	0.17	0.21	0.50	0.09	0.67	0.91
n-6 DPA, 22:5n-6	ω-6	−0.10	0.47	0.83	0.26	0.24	0.68	0.06	0.67	0.81	0.23	0.29	0.91
n-3 DPA, 22:5n-3	ω-3	−0.14	0.30	0.66	0.20	0.36	0.84	0.16	0.24	0.52	0.20	0.36	0.91
DHA, 22:6n-3	ω-3	−0.02	0.88	0.93	0.18	0.41	0.86	0.11	0.43	0.61	0.18	0.42	0.91

[1]DPA, docosapentaenoic acid; FDR, false discovery rate; FFA, free fatty acid. *indicates statistical significance.

influence on cytokine concentrations. If significant associations were identified, a more complex relation between NEFAs, cytokines, and depression was explored using multiple regression.

Results

Description of subjects

Demographic, ethnic, and biobehavioral characteristics were generally similar among cancer patients and caregivers. Of note, mean exercise habits were lowest in cancer patients (**Table 1**). Total depression scores (GRID Ham-D) were also highest in cancer patients. In total, 19 of 56 (33.9%) cancer patients and 3 of 23 (13.0%) caregivers were diagnosed with major depression based on the DSM-IV (SCID) (26). Cancers were of mixed type and stage, with the majority recruited from either the gastrointestinal (38%) or lung cancer clinics (16%) and having late-stage disease (63%).

Nonparametric statistical analysis

No significant differences were found in NEFA concentrations among sample groups after FDR correction (**Table 2**). However, the weight percentage of palmitoleic acid (16:1n–7) and the sum of *cis* + *trans* palmi-

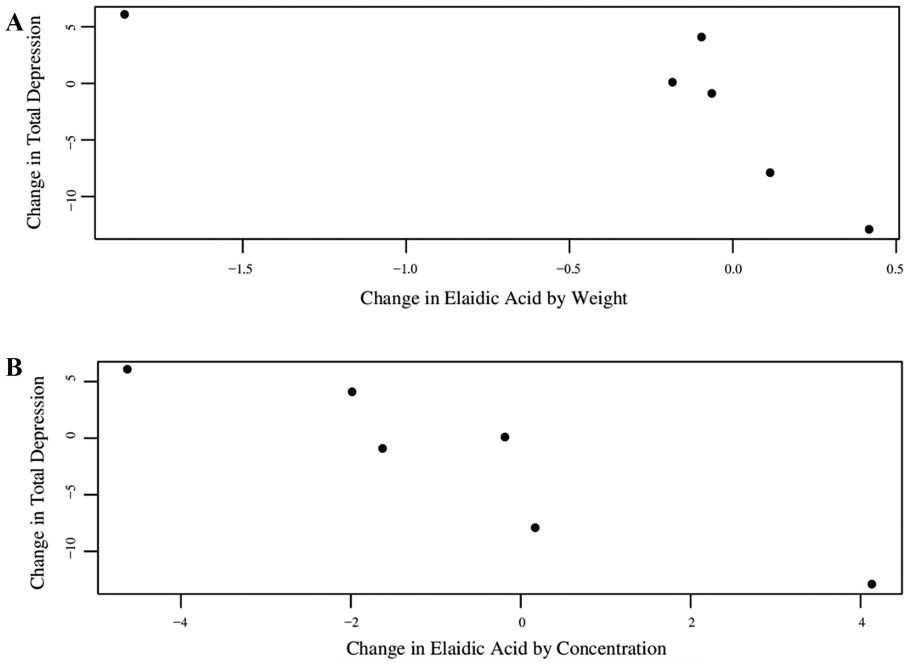

FIGURE 1 Follow-up data on 6 patients provide further support for a negative trend between changes in total depression and elaidic acid by weight percentage (A) and concentration (B). Patients exhibiting higher elaidic acid concentrations, relative to earlier time points, also exhibited lower depression levels.

TABLE 5 Selected features and associated *P* values from regularized regression analysis in cancer patients[1]

Weight %				Concentration			
Feature	**Type**	**Coefficient**	***P***	**Feature**	**Type**	**Coefficient**	***P***
				Major depression			
Nothing selected				Cancer type (lung)**		−0.23	
				Behenic acid, 22:0		−0.18	
				Elaidic acid, *trans*-18:1n-9		−0.10	
				Age**		−0.07	
				Cancer type (gynecologic)**		0.04	
				Total depression			
Elaidic acid, *trans*-18:1n-9		−2.41	0.02	Elaidic acid, *trans*-18:1n-9		−2.59	
Exercise habits**		−1.74		Exercise habits**		−1.80	
Age**		−1.33		Age**		−1.51	0.06
Cancer type (skin)**		1.02		Linoleic acid, 18:2n-6	ω-6	1.29	
Eicosatrienoic acid, 20:3n-3	ω-3	0.81		Cancer type (skin)**		1.26	
Cancer type (breast)**		0.77		Eicosatrienoic acid, 20:3n-3	ω-3	1.20	
Cancer type (gynecologic)**		0.76		Cancer type (gynecologic)**		0.96	
Stearic acid, 18:0		−0.61		Lauric acid, 12:0		0.91	
Myristic acid, 14:0		−0.55		Palmitic acid, 16:0		0.71	
Lauric acid, 12:0		0.53		Vaccenic acid, 18:1n-7		0.60	
Caprylic acid, 8:0		−0.37		Cancer type (breast)**		0.53	
Gender (female)**		0.34		Myristic acid, 14:0		-0.48	
Vaccenic acid, 18:1n-7		0.34		Gender (female)**		0.37	
Late cancer stage**		−0.04		Cancer type (unknown primary)**		0.14	
				EPA, 20:5n-3	ω-3	0.08	
				Late cancer stage**		−0.07	
				Myristoleic acid, 14:1n-5		0.01	

[1]Logistic regression with major depression (top), and linear regression with total depression (bottom) are shown for both NEFA weight % and concentration. Covariates (age, exercise, gender, and cancer characteristics) are indicated with **. *P* values < 0.10 are indicated. DPA, docosapentaenoic acid; NEFA, nonesterified fatty acid.

TABLE 6 Selected features and *P* values from regularized regression in caregivers[1]

Weight %				Concentration			
Feature	**Type**	**Coefficient**	***P***	**Feature**	**Type**	**Coefficient**	***P***
Age**		−0.66	<0.01	Age**		−0.71	<0.01
Exercise habits**		0.27		Exercise habits**		0.25	
Gondoic acid, 20:1n-9		0.13		Nervonic acid, 24:1n-9		0.16	
Nervonic acid, 24:1n-9		0.10		Gondoic acid, 20:1n-9		0.13	
Arachidonic acid, 20:4n-6	ω-6	0.07		Lauric acid, 12:0		0.03	
Lauric acid, 12:0		0.05					
n-6 DPA, 22:5n-6	ω-6	0.04					
Behenic acid, 22:0		0.04					

[1]Linear regression with total depression is shown for both NEFA weight % and concentration. Covariates (age, exercise, and gender) are indicated with **. *P* values < 0.10 are indicated. DPA, docosapentaenoic acid; NEFA, nonesterified fatty acid.

TABLE 7 Pearson correlations between log-transformed elaidic acid (weight %) and cytokine concentrations in cancer patients[1]

	ρ	***P***	**FDR**
IL-6	−0.20	0.13	0.49
IFN-γ	−0.17	0.21	0.49
IL-10	−0.05	0.74	0.85
IL-12p70	0.19	0.16	0.49
IL-13	0.14	0.30	0.56
IL-1b	−0.03	0.83	0.85
IL-2	−0.05	0.71	0.85
IL-4	0.17	0.22	0.49
IL-5	0.03	0.81	0.85
IL-8	−0.08	0.57	0.85
TNF-α	−0.08	0.56	0.85
IL-1ra	−0.27	0.05*	0.49
IL-17	−0.03	0.85	0.85
IL-12p40	0.23	0.09	0.49
IL-2ra	−0.16	0.23	0.49

[1]FDR, false discovery rate. *indicates statistical significance.

TABLE 8 Linear regression of log-transformed elaidic acid and IL-1ra on total depression, with an interaction term[1]

	Coefficient	**SE**	***t*-statistic**	***P***
Intercept	10.51	10.44	1.01	0.32
Elaidic acid	14.15	8.29	1.71	0.09
IL-1ra	−0.27	1.34	−0.20	0.84
Elaidic acid:IL-1ra	−2.15	1.06	−2.03	0.05*

[1]*indicates statistical significance.

toleic acid isoforms were significantly different among groups prior to FDR correction, with healthy controls having lower levels than cancer patients and caregivers.

NEFA concentrations were not significantly different between cancer patients with and without categorically defined major depression after FDR correction (**Table 3**). However, several NEFAs were significantly different prior to FDR correction. Both elaidic acid (*trans*-18:1n–9) and behenic acid (22:0) had lower weight percentage and concentration in cancer patients with major depression compared with cancer patients without major depression. Erucic acid (22:1n–9) concentrations were lower in cancer patients with major depression, whereas α-linolenic acid (ALA; 18:3n−3) weight percentage was higher in cancer patients with major depression.

Within cancer patients, several NEFAs were significantly associated with continuous depression severity scores prior to FDR correction, but only elaidic acid remained significant after FDR correction (**Table 4**). Elaidic acid, measured as weight percentage, was negatively correlated with total depression in cancer patients ($\rho = -0.51$, nominal $P < 0.01$; $P = 0.00$ after FDR correction). Prior to multiple test correction, linoleic acid (18:2n–6) and ALA were significantly positively correlated with total depression using both measures of NEFA concentrations (weight percentage and concentration), and numerous other NEFAs were significantly correlated with total depression based on 1 NEFA measure (either weight percentage or concentration). No significant correlations were found in the caregiver sample for weight percentage or concentration after multiple testing correction (Table 4). However, before multiple testing correction, nervonic acid (24:1n–9) was significantly and positively associated with depression when measured as weight percentage and concentration.

Given the significant result for elaidic acid in cancer patients, follow-up data were examined for the 6 cancer patients who had been evaluated at a second time point. A negative trend was identified between the change in elaidic acid and change in depression status at a second time point based on both weight percentage and concentration, consistent with initial results (**Figure 1**). The negative association was significant using Spearman rank correlation ($P < 0.02$ for both weight percentage and concentration).

Multivariate regression analysis.

No significant relations were found between NEFAs and major depression using regularized logistic regression in cancer patients (**Table 5**). However, a significant relation was identified in regularized linear regression between elaidic acid and total depression when measured as weight percentage ($P < 0.02$) but not concentration (Table 5). When NEFAs were measured as concentrations, no significant relations were identified between NEFAs and total depression. Analyses conducted without cancer-specific covariates (cancer type, cancer stage, active treatment) were similar to those conducted that included cancer covariates, with a high degree of overlap in the features selected and similar *P* values (**Supplemental Table 1**). When BMI was included as a covariate with concentration data, BMI was not selected as an important variable in regularized regression, and no NEFAs were significantly related to depression (**Supplemental Table 2**). BMI was not significantly different

between patients with and without major depression ($P = 0.44$) and was not correlated with total depression ($\rho = 0.13$, $P = 0.39$). No significant relations were identified between NEFAs and total depression among caregivers for either weight percentage or concentration (**Table 6**).

NEFAs and cytokines

Given the significant association between elaidic acid and depression in cancer patients, potential associations with cytokines were explored. Elaidic acid was negatively associated only with IL-1ra in Pearson correlation analysis ($P < 0.05$, $\rho = -0.27$), although not after FDR correction (**Table 7**). Regression analyses were then conducted to explore whether more complex relations might exist between elaidic acid and IL-1ra. Single-variable models were compared with multiple-variable models, with and without an interaction term, using the Akaike information criterion (31). The preferred model included a significant interaction between elaidic acid and IL-1ra ($P < 0.05$) (**Table 8**), with the negative relation between elaidic acid and depression being more pronounced when IL-1ra concentrations were high (**Figure 2**).

Discussion

The original hypothesis that pro- and anti-inflammatory NEFAs (specifically ω-3 and ω-6 fatty acids) would influence depression was not well supported in this exploratory study. This may be due to differences in depressive pathophysiology in cancer patients compared with depression without an inflammatory medical comorbidity (23). In general, our study largely found null relations between NEFAs and depression with few differences in NEFA concentrations based on diagnosis. This study identified proinflammatory ω-6 NEFA linoleic acid and the anti-inflammatory ω-3 NEFA ALA as significantly and positively associated with depression in cancer patients (both as weight percentage and concentration), but only before FDR correction. It is important to note that the plasma NEFA pool is highly dynamic with relatively short half-lives (32). Thus, increased concentrations of linoleic acid and ALA could be related to increased dietary intake or to decreased metabolic consumption and further replication and mechanistic studies are warranted.

A more highly significant, but unexpected, result was the negative association between elaidic acid and total depression in cancer patients. Elaidic acid is the major *trans* fatty acid in partially hydrogenated vegetable oils. Within Canada, despite voluntary efforts to remove *trans* fats from the food supply, it has been reported that bakery products and dairy products and substitutes remain relatively high in *trans* fatty acids (21). While we did not collect dietary information from the subjects in our study, it would be of interest for future studies to examine if the aforementioned foods high in *trans* fats are consumed by cancer patients with depression. *trans* Fats have been positively associated with depression in several dietary studies (19, 20, 33). Elaidic acid has also been shown to increase the expression of proinflammatory cytokines IL-1b and IL-6 in various animal tissues (34). Interestingly, in this study, cancer patients with high concentrations of elaidic acid had lower total depression scores. Reverse causation, which would occur if depressed patients were more likely than nondepressed patients to consume elaidic acid, is a possible explanation of this finding. In other words, these

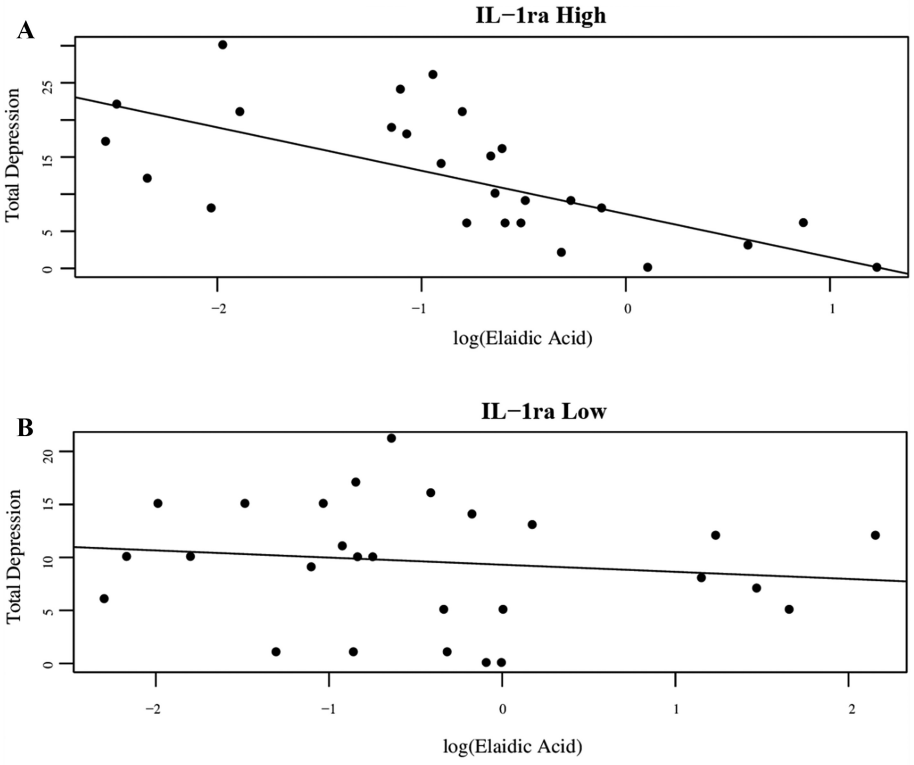

FIGURE 2 Relationship between elaidic acid and total depression at high (> median) (A) and low (< median) (B) values of IL-1ra.

results may simply reflect the different eating habits of depressed patients as compared with nondepressed patients. Nevertheless, given the strength of the negative association, it is possible that elaidic acid in fact plays a beneficial role with respect to depression.

Elaidic acid is the only ligand to selectively activate the free fatty acid receptor 1 (FFAR1), also known as the G protein–coupled receptor 40 (GPR40) (35). GPR40 is widely expressed in the pancreas and the brain, and binding of elaidic acid to GPR40 induces a robust transient elevation of calcium concentrations (35). GPR40 activation by the synthetic agonist GW9508 (36) has been associated with synaptic plasticity (37), a reduction in depressive behavior (38), and a reduction in pain symptoms and inflammation in mice (39). Moreover, knock-down experiments demonstrate mice lacking GPR40 protein receptors had higher pain and inflammation (40, 41), and mice injected with inflammatory agents increased GPR40 expression compared with a control group (39). The underlying mechanisms are not well elucidated, but they may involve an upregulation of β-endorphins or regulation of cytokines (39).

Furthermore, GW9508 was found to reduce pain symptoms in inflamed mice, but not in the naïve controls (39), suggesting that upregulation of GPR40 may amplify the pain-reduction mechanism. Although preliminary, our finding that elaidic acid was significantly associated with depression in cancer patients, but not in caregivers, is consistent with the hypothesis that higher inflammatory levels in cancer patients may make them more sensitive to the effects of elaidic acid. The finding that the negative relation between elaidic acid and depression was stronger when IL-1ra concentrations were high can also be interpreted in this way, as IL-1ra is considered a surrogate biomarker for proinflammatory IL-1 (42) and was also linked to continuous depression severity in the current study population (23). IL-1ra is an anti-inflammatory protein, counter-regulating the effects of IL-1, potentially working through the CIRS (8, 42).

This study has limitations, including lack of information regarding the diets of patients in both arms, the exploratory nature of the work, and the low number of categorically depressed caregivers. Furthermore, this study was constrained to relatively small sample sizes, which may have limited the statistical power to detect associations with ω-6 fatty acids and depression. The potential mechanisms outlined are preliminary and further research is needed to assess their validity. Study strengths include a focus on a well-described and specific medical population with previously characterized cytokine concentrations, a healthy control comparison group, and the novelty of examining the NEFA pool in cancer patients in relation to depression. Moreover, the analytical approach used was designed to reduce overfitting and minimize the number of false discoveries.

This study contributes to an active area of research on the potential interconnections between NEFAs, G protein receptors, and inflammation, and the findings warrant further investigation. A novel hypothesis emerging from this work is a potential role for elaidic acid in alleviating symptoms of depression. If confirmed, the findings could lead to potential dietary interventions for depression in highly inflamed patients.

Acknowledgments

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