UNIVERSITY OF CALIFORNIA SAN DIEGO

Dysregulation of Fatty Acids and Related Metabolites in Women with Anorexia Nervosa

A thesis submitted in partial satisfaction of the requirements
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by

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The thesis of Nhien Ngoc Quynh Nguyen is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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The Introduction, Subjects and Methods, sections 3.1-3.2, and Discussion, in part, are a reprint of the material as it appears in *Food-Intake Normalization of Dysregulated Fatty Acids in Women with Anorexia Nervosa*. Nguyen, Nhien; Dow, Michelle; Woodside, Blake; German, J. Bruce; Quehenberger, Oswald; Shih, Pei-an Betty, Nutrients, 2019. The thesis author was the first author of this paper.

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ABSTRACT OF THE THESIS

Dysregulation of Fatty Acids and Related Metabolites in Women with Anorexia Nervosa

by

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Master of Science in Biology

University of California San Diego, 2020

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Professor Gen-sheng Feng, Co-Chair

Anorexia nervosa (AN) is an eating disorder characterized, in part, by aversion toward high-fat foods. Foods that are high in fats are major sources of fatty acids (FAs). FAs are crucial for brain health and have shown to be altered in psychiatric illnesses including eating disorders. The n-3 and n-6 FAs can

undergo auto-oxidation or catalytic conversions in enzymatic pathways such as lipoxygenases (LOX), cyclooxygenases (COX), and cytochromes P450 (CYP) to form oxygenated metabolites termed oxylipins. Oxylipins formed by the Cytochrome P450 (CYP) enzymes are epoxy fatty acids, which are converted into diol fatty acids by soluble epoxide hydrolase (sEH). The genetic variants in the gene that encodes sEH, *EPHX2*, have been shown to be associated with AN risk. We studied the role of FAs and sEH pathway in AN using 50 AN cases and 47 control women. We have identified abnormal concentrations of several fatty acids at both fasting and postprandial timepoints in AN, a trend of elevated sEH activity and expression, and significant differences in several sEH-associated oxylipins in AN. These results confirm the n-3 PUFA-driven dysregulation in plasma PUFA, elevation of sEH in vivo and ex vivo, and aberrant oxylipins in AN. More studies with larger sample sizes are needed to confirm these findings and examine the mechanisms by which these dysregulations affect AN pathogenesis.

1. Introduction

1.1. Anorexia Nervosa

Anorexia nervosa (AN) is an eating disorder characterized by body image disturbances and extreme dietary restrictions [1]. AN affects 0.3% to 4% of women [2-4] and is the deadliest psychiatric disorder with mortality rates ranging from 4.4% to 18% [4, 5]. The current treatments for AN are not consistently effective; patients with AN are known to resist treatments [6] and have a high relapse rate ranging from 11% to 36% [7-10]. The pathophysiology of AN is influenced by interactions between environmental, psychological, and biological factors [2, 11, 12]. However, the biological etiology of AN remains unclear. A better understanding of the biological underpinning of AN is needed to improve treatment effectiveness and reduce relapse risk for individuals with AN.

AN patients have an intense fear of gaining weight and practice extreme dietary restraints in pursuit of thinness [1]. Compared to healthy controls, women with AN prefer low-fat diets [13] and have a strong aversion toward high-calorie, high-fat foods [14, 15]. Decreased quantity of total fats in the diet has been linked to longer illness duration in AN [13]. Moreover, AN patients who consume lower amount of dietary fat exhibited poorer treatment outcomes compared to AN patients who consumed a normal amount of dietary fat. [16]. Abnormal fat absorption and metabolism have been suggested as factors underlying homeostatic disruptions in AN [17]. These findings indicate that fat aversion and reduced fat intake may be linked to metabolic alterations that contribute to AN etiology. Thus, investigating the biological and phenotypic changes related to consumption of a high fat meal may provide important data to elucidate AN etiology and improve future treatments.

1.2. Fatty Acid Abnormalities in Anorexia Nervosa

The high-fat foods that AN patients avoid are major dietary sources of fatty acids (FAs). FAs can be classified into saturated FAs, which do not have any double bonds, and unsaturated FAs, which contain one (monounsaturated) or more (polyunsaturated) double bonds [18, 19]. Unsaturated FAs can be

categorized into n-3, n-6, n-7, and n-9 FAs, which are found in many food-sourced oils such as fish oil (n-3), soybean oil (n-6), butter (n-7), and olive oil (n-9) [20-23]. These FAs, especially the well-studied n-3 FAs, are crucial for healthy brain development, as altered levels of n-3 FAs have been implicated in various psychiatric disorders including schizophrenia, depression, and anxiety [24, 25]. Plasma n-3 FAs were found to be significantly elevated in AN patients compared to controls in several studies, including a previously published study from our lab [26, 27]. However, two other studies reported reductions in long-chain n-3 FAs in plasma phospholipids and triglycerides in young women with AN [28] and in erythrocyte membranes among female adolescents with eating disorders [29]. In comparison, AN patients showed lower proportions of n-6 FAs in several plasma lipids (phosphatidylcholine, triglycerides, and cholesteryl esters) in one study [30], and in plasma phospholipids in another study [31]. The monounsaturated hexadecenoic acid, oleic acid, and vaccenic acid were found to be increased in AN compared to healthy controls [30]. Saturated FAs were also altered in AN; one study showed reduced proportions of plasma stearic acid [31] while other studies reported increases in total saturated FAs [28], stearic acid, and palmitic acid in AN [27]. Discrepant findings on FA patterns in eating disorders, specifically AN, can result from differences in the studied populations (in terms of age, illness duration, etc.) and variation in assessment methodology and study design. The well-known importance of FAs in brain health and evidence of FA alterations in AN indicate a likely involvement of FAs in AN pathophysiology.

1.3. Soluble Epoxide Hydrolase and Polyunsaturated Fatty Acid Metabolites

n-3 and n-6 polyunsaturated FAs (PUFAs) can undergo auto-oxidation or enzymatic pathways such as cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) to form oxygenated metabolites termed oxylipins, which are bioactive lipid mediators involved in many biological processes. CYP-derived metabolites of n-3 and n-6 PUFAs are known as epoxy fatty acids (epoxides). These epoxy fatty acids (e.g., ARA-derived 11,12-EpETre) can be further converted into dihydroxy fatty acids (diols) (e.g., 11,12-DiHETrE) by soluble epoxide hydrolase (sEH) [32-35]. sEH is an enzyme downstream of the CYP pathway that is present in both peroxisomes and cytosol [36] and can be found in many organs,

including the brain, intestine, liver, and kidney [37]. sEH has been reported to play important roles in various biological processes and in disorders including anorexia nervosa [38, 39]. Genetic variation in *EPHX2*, the sEH-coding gene, was found to be associated with AN risk, and in vivo activity of sEH was elevated in AN compared to healthy controls [26]. These findings indicate that in vivo activity of sEH may be an important risk factor for AN.

1.4. The Inflammatory Hypothesis

Inflammation has been implicated in various psychiatric disorders, such as depression, bipolar disorder, and schizophrenia [40-43]. While a few studies did not show strong evidence for high inflammatory markers in AN patients [44, 45], two meta-analyses reported elevated levels of pro-inflammatory cytokines in patients with eating disorders, specifically AN, indicating a role of inflammation in AN [46, 47]. Elevated inflammation in AN may be modulated by sEH via its biological actions on epoxy fatty acids. The epoxy fatty acids that sEH uses as substrate have been shown to exert anti-inflammatory and neuroprotective properties [39, 48-51]. In comparison, the diol fatty acids, produced from the catalytic function of sEH, are less active forms compared to their epoxy precursors [39, 52-54]. As a result, the formation of these diol fatty acids leads to a more inflammation-modulatory state, including stimulation of monocyte chemotaxis [55]. Given the association between the gene encoding sEH and AN. the role sEH plays in inflammation regulation, and the involvement of inflammation in psychiatric disorders such as AN, we hypothesized that sEH pathway plays a key role in the AN risk.

It is currently unclear how FAs, their metabolites, and their regulatory enzymes affect AN pathophysiology and if they could serve as useful biomarkers for AN. To elucidate the role of FAs and oxylipins in AN, we applied a multi-omics (i.e., lipidomics and metabolomics) approach to compare levels of FAs, oxylipins, and sEH between women with AN and healthy control women under both fasting and postprandial conditions. We aim to first confirm the previously reported associations between FAs and AN [26]. Additionally, we employed a food-challenge protocol to assess if FAs and their metabolites (oxylipins) are associated with AN risk and clinical phenotypes. We hypothesized that AN patients will display altered

plasma FA concentrations as well as differential sEH activity/expression and resulting oxylipin levels compared to control women. Clarifying the relationship between sEH pathway markers in AN will help to elucidate AN etiology, enable new biomarker identification, and pave the way for more effective prevention and treatment programs for AN.

The Introduction, in part, is a reprint of the material as it appears in *Food-Intake Normalization of Dysregulated Fatty Acids in Women with Anorexia Nervosa*. Nguyen, Nhien; Dow, Michelle; Woodside, Blake; German, J. Bruce; Quehenberger, Oswald; Shih, Pei-an Betty, Nutrients, 2019. The thesis author was the first author of this paper.

2. Subjects and Methods

2.1. Study Design and Human subjects

While the recruitment of the study is ongoing, this thesis utilized a subset of enrolled study participants for analyses described within. Participants were recruited from the University of Toronto and the University of California, San Diego (UCSD). Data collected from 50 women with AN [30 ill AN (age 29.6 ± 9.0) and 20 recovered AN (age 31.1 ± 12.0)] and 47 healthy control women (age 30.5 ± 9.3) who have been enrolled in the study were used for this thesis. Blood samples and research questionnaires were collected from all study subjects after at least 10 hours of fasting. Participants then ate the study challenge meal consisting of sausage, egg, cheese, and English muffins (Calories: 436; fat: 27 g [saturated fat =11 g]; carbohydrates: 28 g; protein: 19 g). Two hours after participants finished the sandwich, blood samples and questionnaires were obtained from 28 women with AN (9 ill and 19 recovered AN) and 47 healthy control women. 22 subjects in the AN group refused the challenge meal for reasons including being “too sick to eat” (n = 13, all AN) and abstaining from meat or pork products (n = 9).

Prospective subjects interested in the study contacted recruitment site investigators and underwent a phone interview to assess their eligibility and receive further information about the study. Participants with AN had to be formally diagnosed with AN by a licensed psychiatrist. Exclusion criteria for all

prospective subjects included having an Axis I psychiatric illness, organic brain syndrome, schizophrenia or schizoaffective disorder, untreated thyroid disease, renal disease, hepatic disease, being pregnant or breast feeding, or using fish oil supplements on a regular basis. Subjects who reported regular use of fish oil supplements and were willing to discontinue fish oil supplementation for 30 days prior to the study visit remained eligible for the study if all other eligibility criteria have been met. This study has been approved both by the UCSD Human Protection Board and the University of Toronto Research Ethics Board.

2.2. Analytical Methods: Fatty Acids, Soluble Epoxide Hydrolase, and Oxylipins

The plasma FA concentrations were measured by gas chromatography-mass spectrometry (GC-MS) at the UCSD Lipidomics Core as previously reported [26, 56, 57]. Briefly, to analyze the total (sum total of esterified and non-esterified) plasma FA composition, human plasma (10 uL) in methanol (250 uL) was supplemented with a cocktail of internal standards consisting of 15 deuterated FAs, saponified with 0.5 N KOH for 30 min at 37 °C and then adjusted to pH = 4 with glycine buffer. The FAs were extracted with isooctane and derivatized with pentafluorobenzyl (PFB) bromide. The resulting fatty acid PFB esters were analyzed by GC-MS using a negative chemical ionization mode (Agilent 6890N gas chromatograph equipped with an Agilent 5973 mass selective detector; Agilent, Santa Clara, CA, USA). All reagents and solvents were of highest purity, suitable for mass spectral analyses and were purchased from ThermoFisher Scientific (Waltham, MA, USA). All fatty acid standards (purity > 99%) used for identification and quantification were purchased from Nu-Chek Prep Inc. (Elysian, MN, USA). All deuterated fatty acids that were used as internal standards were purchased from Cayman Chemical (Ann Arbor, MN, USA). Standard curves for each of the FAs were acquired in parallel using identical conditions. The quantitative assessment of FAs in a sample was achieved by comparison of the mass spectrometric ion signal of the target molecule normalized to the internal standard with the matching standard curve according to the isotope dilution method. The concentrations of the FAs are reported in pmol/mL.

Proxy markers for activities of fatty acid desaturases and elongases were formed as followed: For stearoyl-CoA desaturase-16 (SCD-16): palmitoleic acid/palmitic acid [58-60]; for stearoyl-CoA desaturase-

18 (SCD-18): oleic acid/stearic acid [58, 60]; for delta-5-desaturase (D5D): arachidonic acid (ARA)/dihomo-gamma-linoleic acid (DGLA) [58-60]; for delta-6-desaturase (D6D): stearidonic acid/alpha-linoleic acid (ALA) [61], gamma-linoleic acid (GLA)/linoleic acid (LA) [58, 59], and DGLA/LA [60]; for elongase 2 (ELOVL2): adrenic acid/ARA [62]; for elongase 5 (ELOVL5): DGLA/GLA [58]; and for elongase 6 (ELOVL6): stearic acid/palmitic acid [58].

sEH expression and activity were analyzed using peripheral blood mononuclear cells (PBMCs) from five women with AN (age 25.4 ± 5.5; two Ill AN and three Recovered AN) and five healthy control women (age 24.8 ± 5.9) during both fasting and postprandial states. sEH expression (ng/mL) was quantified using a sandwich ELISA format using a rabbit polyclonal IgG as the capture antibody and a nanobody as the reporting antibody. sEH activity (pmol/mL/min was measured using a radioactive assay that employed T-DPPO, a radioactive substrate that was prepared using a new proprietary synthetic method.

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used to measure plasma concentrations of 11 epoxy fatty acids and 11 corresponding diol fatty acids from the same subset of subjects whose sEH expression and activity were quantified (five women with AN and five healthy controls). Agilent 1200 SL liquid chromatography series (Agilent Corp., Palo Alto, CA, USA) was used for liquid chromatography. Liquid chromatography was performed on an Eclipse Plus C18 2.1×150 mm, 1.8-μm column (Agilent Corp.), with the autosampler kept at 4 °C. Mobile phase A consisted of water with 0.1% glacial acetic acid. Mobile phase B contained acetonitrile/methanol (84/16) and 0.1% glacial acetic acid. Gradient elution was performed at a flow rate of 250 μl/min. Chromatography was optimized to separate all analytes in 21.5 min. Analytes were then eluted based on their polarity. The most polar analytes, prostaglandins, and leukotrienes, eluted first, followed by hydroxy and epoxy fatty acids. The instrument was operated in negative multiple-reaction monitor mode. The column was connected to a 4000 QTrap tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray source (Turbo V). LC-MS/MS protocols were the same as previously described [63]. Quality control samples were analyzed at a frequency of at least 10 h to ensure stability of the analytical calibration. Analyst

software 1.5.1 was used to quantify oxylipins according to standard curves. Fasting and postprandial concentrations of the these oxylipins are reported in nmol/L.

Eleven sEH diol product/epoxy fatty acid substrate ratios were used as in vivo markers of sEH activity: ARA-derived 8,9-DiHETrE/8,9-EpETrE, 11,12-DiHETrE/11,12-EpETrE, and 14,15-DiHETrE/14,15-EpETrE; LA-derived 9,10-DiHOME/9,10-EpOME and 12,13-DiHOME/12,13-EpOME; ALA-derived 9,10-DiHODE/9,10-EpODE, 12,13-DiHODE/12,13-EpODE, and 15,16-DiHODE/15,16-EpODE; docosahexaenoic acid (DHA)-derived 19,20-DiHDPE/19,20-EpDPE and 16,17-DiHDPE/16,17-EpDPE; eicosapentaenoic acid (EPA)-derived 17,18-DiHETE/17,18-EpETE [26, 64-66].

2.3. Statistical Analysis

Phenotype data (age, body mass index [BMI], Becker depression inventory [BDI], and Becker anxiety inventory [BAI]) were tested for normality using histograms and Shapiro-Wilk test of normality in R 3.5.2. Wilcoxon rank sum was used to compare study subject characteristics and psychiatric phenotypes between control group and each of the AN groups (ill AN [IAN], recovered AN [RecAN], all AN combined [all AN]). Pairwise similarity between FAs was determined using Pearson correlations between concentration of a pair of FAs, then plotted using seaborn clustermap in Python with row or column clustering set to False. Python was used to run t-tests with false discovery rate (FDR) adjustment to compare plasma concentrations of 26 FAs in AN versus controls at both fasting and postprandial timepoints. Spearman's rho was used to assess correlations between individual FAs and phenotypes (BMI, BDI, BAI, and postprandial change in BAI). T-test was used to compare FA product/substrate ratios as well as n-6:n-3 ratios between AN and controls. Wilcoxon rank sum was used to analyze differences in sEH expression and activity, oxylipin levels, and in vivo sEH activity indices. Pearson correlation analyses were performed to examine the associations between sEH expression, activity, and diol/epoxy fatty acid ratios (surrogate markers of in vivo sEH activity). Multiple regression analyses with age, BMI, sEH protein expression or activity as predictor variables and each of the 11 diol/epoxy fatty acid ratios as the response variable were

generated to study the effects of sEH protein expression or activity on its in vivo activity while controlling for age and BMI.

Subjects and Methods, in part, is a reprint of the material as it appears in *Food-Intake Normalization of Dysregulated Fatty Acids in Women with Anorexia Nervosa*. Nguyen, Nhien; Dow, Michelle; Woodside, Blake; German, J. Bruce; Quehenberger, Oswald; Shih, Pei-an Betty, Nutrients, 2019. The thesis author was the first author of this paper.

3. Results

3.1. Participant Characteristics and Phenotypes

No significant difference in age was found between AN and healthy controls (p-value = 0.71) (Table 1). The mean BMI in controls was 21% higher than the mean BMI in all AN subjects (22.9 ± 3.5 vs. 18.9 ± 3.8, respectively; p-value < 0.01), 37% higher than ill AN (IAN) subjects (22.9 ± 3.5 vs. 16.7 ± 2.4, p-value < 0.001) and 3% higher than recovered AN (RecAN) subjects (22.9 ± 3.5 vs. 22.3 ± 2.8, p-value = 0.5) (Table 1). Depression score was 4.6 times higher in all AN than that in controls (21.8 ± 16.4 vs. 3.9 ± 7.4; p-value <0.001). When stratified by AN recovery status, depression was more than 6 times higher in IAN and 2 times higher in RecAN compared to controls (IAN: 28.0 ± 16.7; RecAN: 12.6 ± 10.9; controls: 3.9 ± 7.4; p-values < 0.001). At the fasting timepoint, mean anxiety score was 4 times higher in all AN compared to controls (21.5 ± 13.7 vs. 4.3 ± 5.8; p-value < 0.001). The IAN group exhibited 5.2 times higher anxiety score, while RecAN group had 2.3 times higher anxiety score than that of controls (IAN: 26.6 ± 13.1; RecAN: 14.4 ± 11.6; controls: 4.3 ± 5.8; p-values < 0.001) (Table 1). All AN and RecAN groups showed greater reductions in anxiety score two hours after eating the meal when compared to controls (p-values = 0.013 and 0.025 in All AN and RecAN, respectively) (Table 1).

Table 1: Study subject characteristics. Entries are of the form mean ± SD. Statistical comparisons for controls versus each of the three AN groups (all AN, ill AN, and recovered AN) were tested using Wilcoxon rank-sum tests. Statistics: * = 0.01 ≤ p-value < 0.05; ** = p-value < 0.01. BMI: body mass index; BDI: Becker Depression Inventory; BAI: Becker Anxiety Inventory. IAN: Ill anorexia nervosa with BMI ≤ 17.5; RecAN: recovered anorexia nervosa with BMI ≥ 18.5 for longer than one year.

					Statistics		
Characteristic	**All AN (N=50)**	**IAN (N=30)**	**RecAN (N=20)**	**Controls (N=47)**	**All AN to Controls**	**IAN to Controls**	**RecAN to Controls**
Age, year	30.1 ± 10.2	29.6 ± 9.0	31.1 ± 12.0	30.5 ± 9.3	0.710	0.68	0.88
BMI, kg/m^2	18.9 ± 3.8	16.7 ± 2.4	22.3 ± 2.8	22.9 ± 3.5	<0.001**	<0.001**	0.5
BDI	21.8 ± 16.4	28.0 ± 16.7	12.6 ± 10.9	3.9 ± 7.4	<0.001**	<0.001**	<0.001**
Fasting BAI	21.5 ± 13.7	26.6 ± 13.1	14.4 ± 11.6	4.3 ± 5.8	<0.001**	<0.001**	<0.001**
Change in BAI	−3.4 ± 7.5	−5.1 ± 10.9	−2.3 ± 4.5	−1.3 ± 3.3	0.013*	0.109	0.025*

3.2. Fatty Acid Dysregulation in AN

3.2.1. Fatty Acid Profile

At both fasting and postprandial timepoints, more positive correlations were observed among the 6 saturated and 20 unsaturated FAs in healthy controls than in AN patients, suggesting that FA metabolism is altered in AN in both fasting and postprandial states (Figure 1). The correlation patterns show distinguishable differences in FA concentrations also between AN (Figure 1A, B) and controls (Figure 1C, D) across the two time points. At both fasting and postprandial timepoints, controls exhibited stronger positive correlations between n-6 and n-7 FAs when compared to AN. Similarly, subgroups of correlations between n-6, n-7 and saturated FAs were observed in controls but not in AN. Within the AN group, the correlations between the FAs generally became more pronounced after eating (Figure 1A, B). AN showed stronger correlations between n-6 and n-7, as well as n-6 and saturated FAs at the postprandial timepoint compared to the fasting state. In the control group, FA correlations were similar between fasting and

postprandial timepoints (Figure 1C, D), with some stronger/more positive correlations between n-6, n-7, and n-9, as well as between n-9 and saturated FAs in the postprandial state.

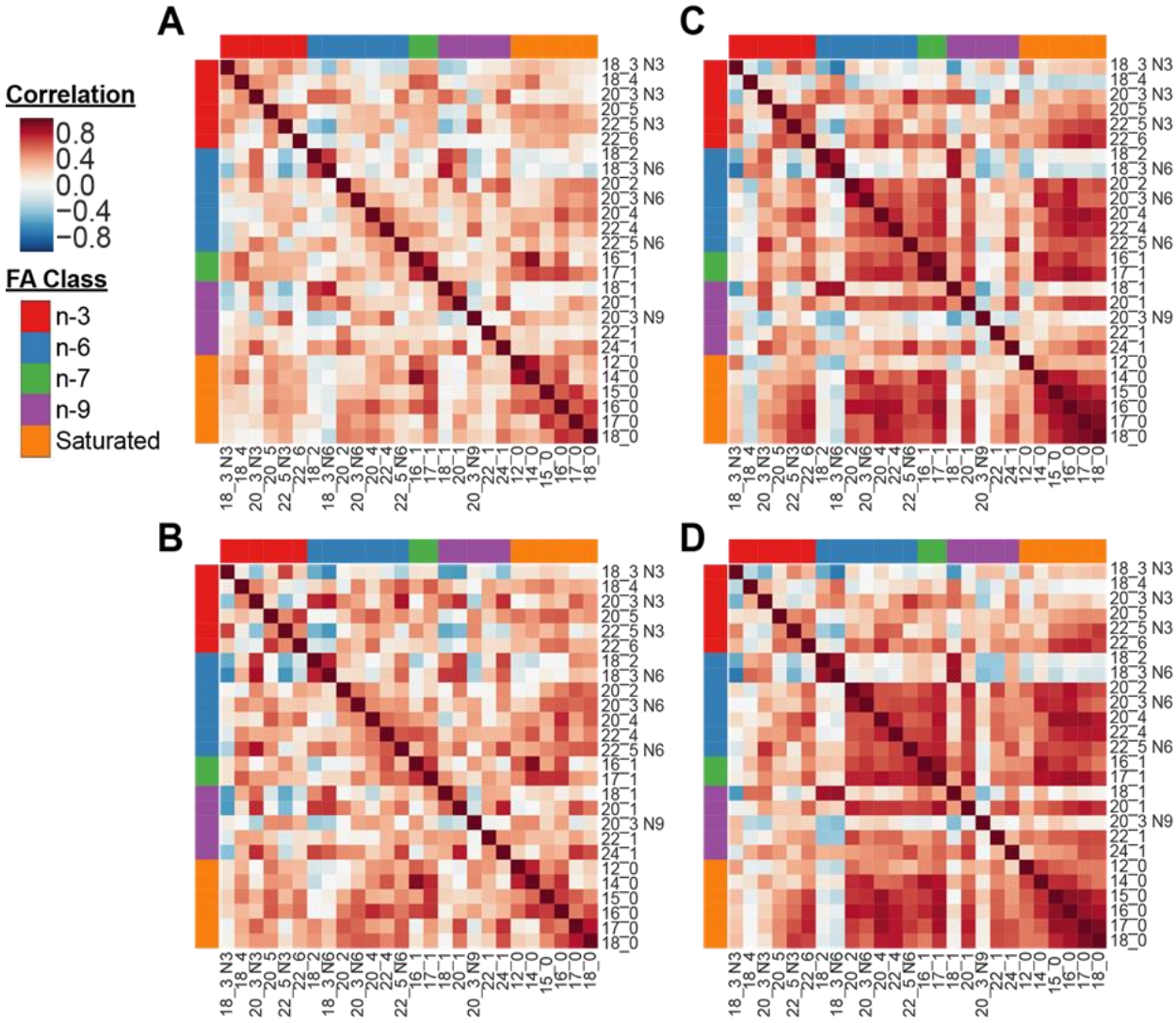


Figure 1: Heatmaps representing pairwise correlations between all fatty acids in anorexia nervosa (**A** [fasting] and **B** [postprandial]) and healthy controls (**C** [fasting] and **D** [postprandial]). Fatty acid classes (n-3, n-6, n-7, n-9, and Saturated) are indicated by the color bars on the top and left side of the heatmaps. In the heatmap, red represented higher correlation, and blue shows lower correlation between a pair of markers.

Out of the 26 FAs examined, four [saturated lauric acid; n-3 ALA, EPA, and docosapentaenoic acid (DPA)] at fasting visit and one (ALA) showed significant increases in AN patients when compared to healthy controls after FDR corrections (Figure 2A). At the fasting timepoint, the saturated lauric acid (12:0) concentration in AN was 1.8 times that in controls (mean ± SD: 15,081.6 ± 14,970.2 pmol/mL and 8,257.4

± 4,740.2 pmol/mL, respectively; p-value = 0.004; FDR-adjusted p-value = 0.023), while the n-3 polyunsaturated ALA (18:3 n-3) in AN was twice the concentration of that in controls (2,217.7 ± 1,587.6 pmol/mL vs. 1,087.9 ± 821.2 pmol/mL; p-value < 0.0001; FDR-adjusted p-value = 0.0009) (Figure 2A). EPA (20:5 n-3) and DPA (22:5 n-3) concentrations among AN were 48% and 56% higher than those in controls (EPA: 33,788.3 ± 17,487.5 pmol/mL vs. 22,860.6 ± 12,642.4 pmol/mL; p-value = 0.0007; FDR-adjusted p-value = 0.006. DPA: 32,664.8 ± 16,215.0 pmol/mL vs. 20,969.0 ± 12,350.0 pmol/mL; p-value = 0.0001; FDR-adjusted p-value = 0.002) (Figure 2A). At the postprandial state, the mean ALA concentration in AN was 1.6 times that of healthy controls (1,830.9 ± 1,115.6 pmol/mL vs. 1,159.4 ± 664.7 pmol/mL; p-value = 0.002; FDR-adjusted p-value = 0.046) (Figure 2B).

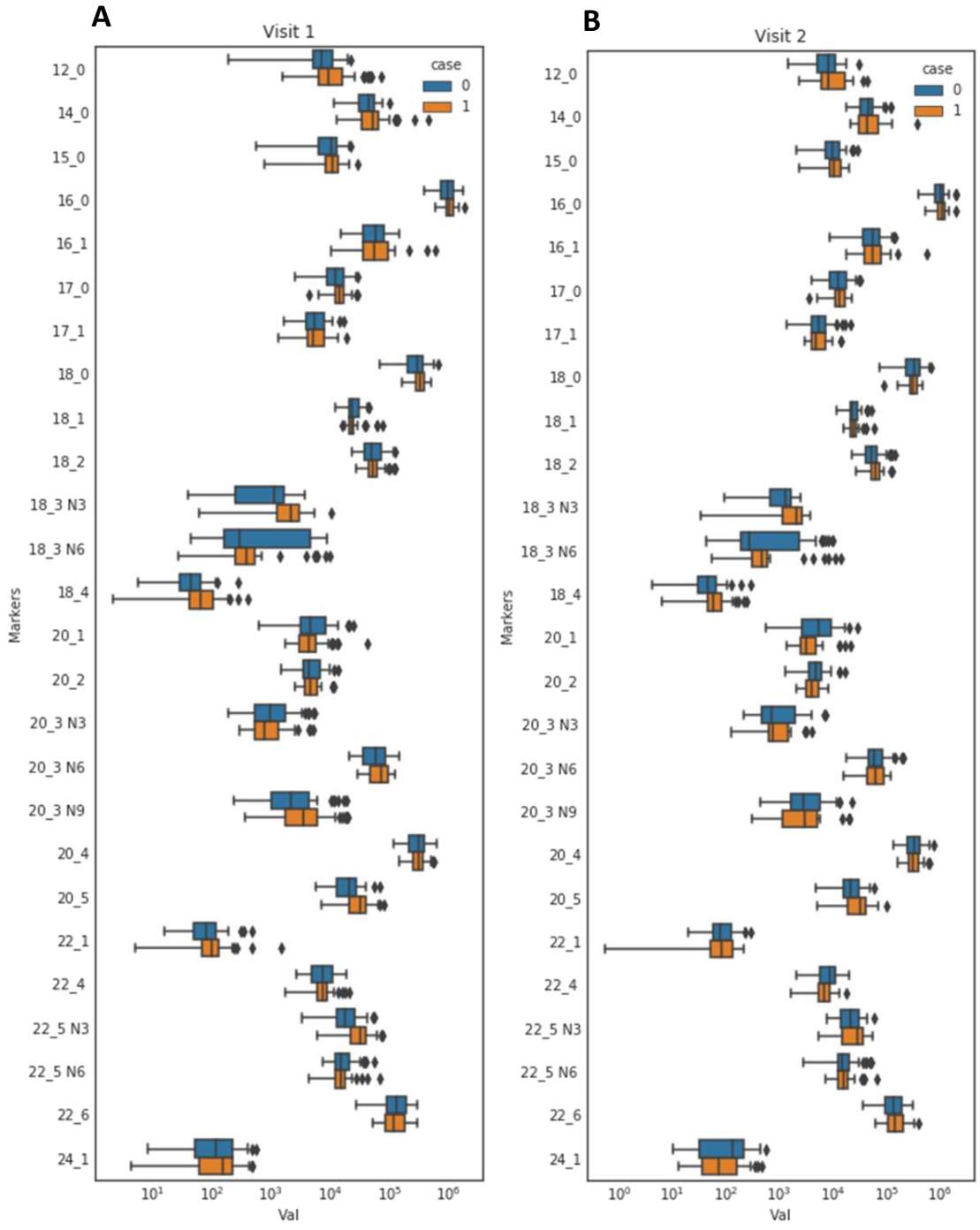


Figure 2: Boxplots representing distribution of each fatty acid in anorexia nervosa group (Orange) and control group (Blue). Orange bars: AN group; blue bars: control group. Visit 1 (**A**) refers to the fasting timepoint, Visit 2 (**B**) refers to the postprandial timepoint (two hours after eating a standardized sandwich). The pairwise similarity was determined using the Pearson correlation between FA concentrations and plotted using seaborn clustermap in Python.

3.2.2. Fatty Acids and AN Phenotypes

The correlations between FAs and AN phenotypes (BMI, depression, fasting anxiety, and change in anxiety after eating) are shown in Table 2 for the fasting timepoint, and Table 3 for the postprandial timepoint. In all subjects, elevation in four FAs (lauric acid, ALA, EPA, and DPA) were significantly correlated with lower BMI in fasting state (Lauric acid: Spearman's rho $r_s = -0.28$; p-value = 0.005. ALA: $r_s = -0.46$, p-value < 0.01. EPA: $r_s = -0.29$, p-value = 0.004. DPA: $r_s = -0.39$, p-value < 0.01) (Table 2). Increased concentrations of fasting n-3 ALA, EPA, and DPA were also significantly associated with higher levels of depression (BDI score) (ALA: $r_s = 0.31$; p-value = 0.002. EPA: $r_s = 0.28$; p-value = 0.005. DPA: $r_s = 0.31$; p-value = 0.002) and higher levels of anxiety (BAI score) (ALA: $r_s = 0.28$; p-value = 0.006. EPA: $r_s = 0.26$; p-value = 0.01. DPA: $r_s = 0.24$; p-value = 0.019) (Table 2). The saturated lauric acid showed no significant correlation with any phenotypes. None of the four FAs were associated with postprandial change in BAI score (Table 2). At the postprandial state, significant inverse associations with BMI remained for lauric acid and ALA, while significant correlations with BDI and BAI also held true for ALA and EPA. A higher concentration of postprandial ALA was associated with a lesser decrease of post-meal anxiety ($r_s = -0.25$; p-value = 0.03) (Table 3).

Table 2: Fasting correlation coefficients of fatty acids with anorexia nervosa phenotypes. Fasting correlation coefficients between fatty acids and phenotypes in all subjects combined, anorexia nervosa group, and control group. Correlation coefficients and p-values were calculated using Spearman's correlation test. BMI: body mass index; BDI: Becker Depression Inventory; BAI: Becker Anxiety Inventory. Statistics: # = 0.05 ≤ p-value < 0.10; * = 0.01 ≤ p-value < 0.05; ** = p-value < 0.01.

Fatty Acids (All Subjects)	**BMI**		**Fasting BDI**		**Fasting BAI**		**Change in BAI**	
	r_s	**p-value**	**r_s**	**p-value**	**r_s**	**p-value**	**r_s**	**p-value**
Lauric acid (12:0 saturated)	−0.28	0.005**	0.15	0.150	0.10	0.350	0.02	0.850
Alpha-linoleic acid (ALA) (18:3 n-3)	−0.46	<0.001**	0.31	0.002**	0.28	0.006**	−0.04	0.744
Eicosapentaenoic acid (EPA) (20:5 n-3)	−0.29	0.004**	0.28	0.005**	0.26	0.010*	−0.12	0.312
Docosapentaenoic acid (DPA) (22:5 n-3)	−0.39	<0.001**	0.31	0.002**	0.24	0.019*	0.03	0.785
Fatty Acids (AN Group)								
Lauric acid (12:0 saturated)	−0.27	0.062#	0.17	0.251	0.04	0.765	−0.15	0.446
Alpha-linoleic acid (ALA) (18:3 n-3)	−0.33	0.019*	−0.03	0.832	−0.09	0.534	0.08	0.684
Eicosapentaenoic acid (EPA) (20:5 n-3)	−0.11	0.441	−0.02	0.900	−0.14	0.318	0.03	0.876
Docosapentaenoic acid (DPA) (22:5 n-3)	−0.30	0.032*	0.27	0.060#	0.05	0.727	0.05	0.782
Fatty Acids (Control Group)								
Lauric acid (12:0 saturated)	−0.15	0.318	−0.11	0.474	−0.15	0.326	0.14	0.347
Alpha-linoleic acid (ALA) (18:3 n-3)	−0.23	0.116	<0.01	0.980	−0.03	0.840	0.10	0.493
Eicosapentaenoic acid (EPA) (20:5 n-3)	−0.20	0.175	0.21	0.162	0.26	0.080#	−0.10	0.498
Docosapentaenoic acid (DPA) (22:5 n-3)	−0.24	0.111	−0.06	0.690	−0.03	0.843	0.12	0.427

In AN only, BMI had significant inverse correlations with fasting n-3 ALA and DPA (ALA: r_s = −0.33; p-value = 0.019. DPA: r_s = −0.3; p-value = 0.032) and marginal inverse correlation with lauric acid (r_s = −0.27; p-value = 0.062) (Table 2). On the contrary, controls exhibited no significant correlations between BMI and any of the four FAs (Table 2). In both AN and control groups, no significant associations between FAs were found with fasting depression, fasting anxiety, or postprandial change in anxiety (Table 2). However, there was evidence for positive correlation between fasting DPA and depression in AN (r_s = 0.27; p-value = 0.060) and between fasting EPA and anxiety in controls (r_s = 0.26; p-value = 0.080) (Table 2). At the postprandial timepoint, BMI was inversely correlated with lauric acid in AN (r_s = −0.36; p =

0.058) and with ALA in controls ($r_s = -0.27$; $p = 0.074$) (Table 3). Increased postprandial concentrations of ALA and EPA were associated with higher scores of depression and anxiety in controls (ALA with depression: $r_s = 0.28$; p-value = 0.060. ALA with anxiety: $r_s = 0.34$; p-value = 0.022. EPA with anxiety: $r_s = 0.33$; p-value = 0.026) but not in AN (p-values > 0.10) (Table 3).

Table 3: Postprandial correlation coefficients of fatty acids with anorexia nervosa phenotypes. Postprandial correlation coefficients between fatty acids and phenotypes in all subjects combined, anorexia nervosa group, and control group. Correlation coefficients and p-values were calculated using Spearman's correlation test. BMI: body mass index; BDI: Becker Depression Inventory; BAI: Becker Anxiety Inventory. Statistics: # = 0.05 ≤ p-value < 0.10; * = 0.01 ≤ p-value < 0.05; ** = p-value < 0.01.

Fatty Acids (All Subjects)	**BMI**		**Fasting BDI**		**Postprandial BAI**		**Change in BAI**	
	r_s	**p-value**	**r_s**	**p-value**	**r_s**	**p-value**	**r_s**	**p-value**
Lauric acid (12:0 saturated)	−0.27	0.018*	0.06	0.640	0.10	0.408	0.06	0.592
Alpha-linoleic acid (ALA) (18:3 n-3)	−0.26	0.027*	0.28	0.016*	0.24	0.037*	−0.25	0.030*
Eicosapentaenoic acid (EPA) (20:5 n-3)	−0.15	0.191	0.25	0.033*	0.28	0.017*	−0.09	0.435
Docosapentaenoic acid (DPA) (22:5 n-3)	−0.17	0.198	0.15	0.198	0.16	0.183	−0.10	0.393
Fatty Acids (AN Group)								
Lauric acid (12:0 saturated)	−0.36	0.058#	0.06	0.764	<0.01	0.99	0.05	0.812
Alpha-linoleic acid (ALA) (18:3 n-3)	−0.14	0.49	0.01	0.959	−0.08	0.702	−0.1	0.602
Eicosapentaenoic acid (EPA) (20:5 n-3)	0.12	0.544	0.09	0.648	−0.04	0.854	0.01	0.979
Docosapentaenoic acid (DPA) (22:5 n-3)	<0.01	0.982	0.14	0.48	0.03	0.866	−0.16	0.403
Fatty Acids (Control Group)								
Lauric acid (12:0 saturated)	−0.16	0.294	−0.04	0.806	0.05	0.746	0.11	0.477
Alpha-linoleic acid (ALA) (18:3 n-3)	−0.27	0.074#	0.28	0.060#	0.34	0.022*	−0.14	0.358
Eicosapentaenoic acid (EPA) (20:5 n-3)	−0.22	0.135	0.23	0.119	0.33	0.026*	−0.02	0.875
Docosapentaenoic acid (DPA) (22:5 n-3)	−0.21	0.157	0.09	0.545	0.15	0.332	0.01	0.941

3.2.3. Differential Indices of Fatty Acid Desaturases and Elongases

Product/precursor ratios of FAs are used as proxy markers of in vivo fatty acid desaturase and elongase enzyme activities (SCD-16, SCD-18, D5D, D6D, and ELOVL [2, 5, and 6]). These ratio markers were compared between AN and controls to explore whether FA regulatory enzymes contribute to FA associations with AN. In the fasting state, three proxy markers were significantly different between AN group and control group (Figure 3A). The marker for SCD-18 and two markers for D6D (represented by n-3 FAs and n-6 FAs) were decreased in all AN compared to controls (SCD-18: AN: 0.08 vs. controls: 0.12; p-value = 0.04; D6D (n-3): AN: 0.08 vs. controls: 0.14; p-value = 0.05; D6D (n-6): AN: 0.02 vs. controls: 0.03; p-value = 0.04) (Figure 3A). Comparison of IAN and control groups showed a more significant reduction of SCD-18 in IAN (0.07 vs. 0.12; p-value = 0.008 (Figure 3A). In the postprandial state, none of the three activity markers remained significantly different between AN and controls (Figure 3B). However, SCD-16 index was noticeably higher in IAN compared to controls (IAN: 0.08 vs. controls: 0.06; p-value = 0.07) (Figure 3B).

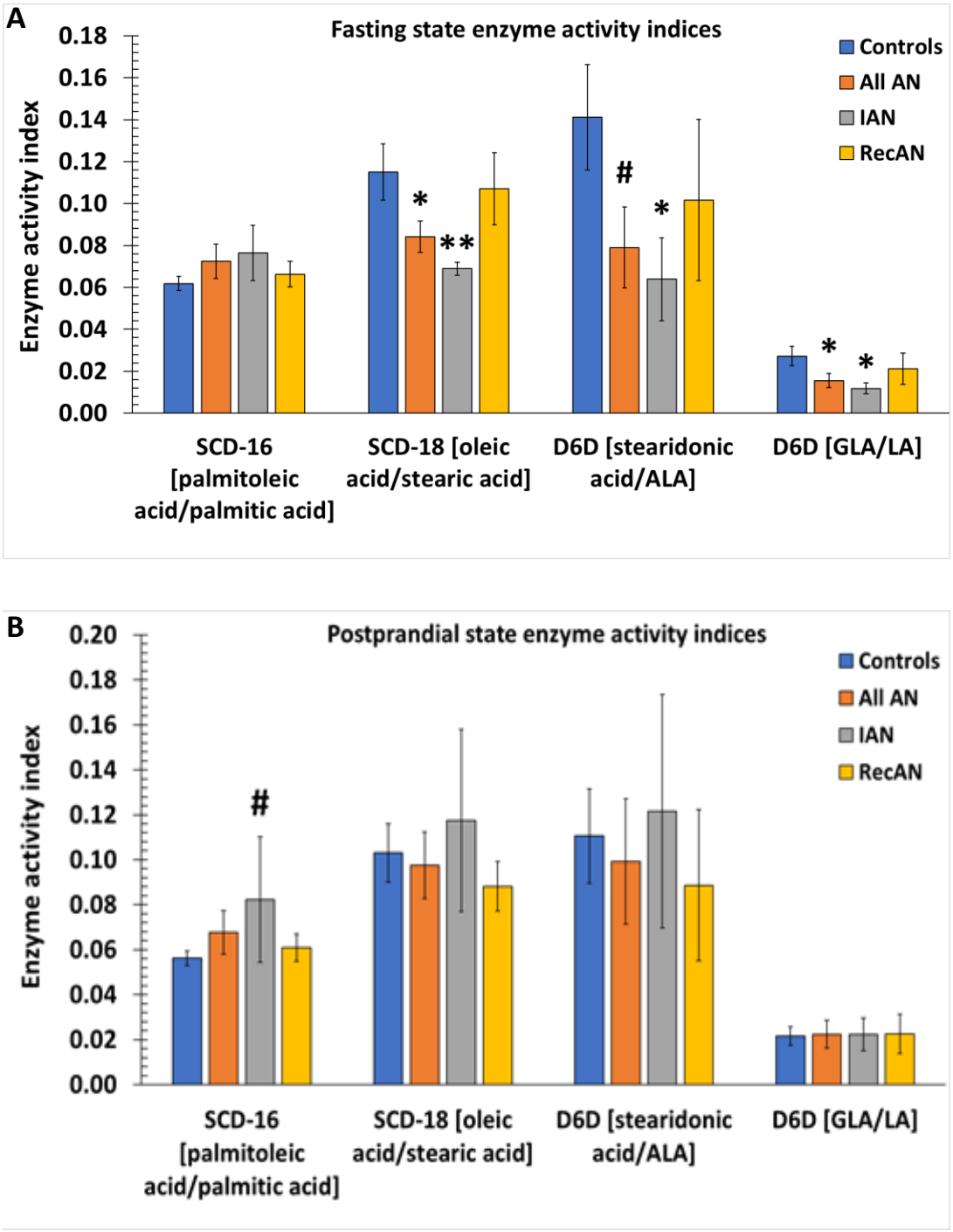


Figure 3: Markers of desaturase and elongase indices and their association with AN status. Fasting (**A**) and postprandial (**B**) enzyme activity indices estimated by fatty acid ratios. Entries are ratios formed by concentrations of individual fatty acids. Fatty acid has unit of measurement of pmol/mL. Bars and error bars represent mean and standard error of the mean. T-test was used to compare mean ratio markers for each individual group of AN (All AN, ill AN, recovered AN) with controls. Statistics: # = $0.05 \leq$ p-value < 0.10; * = $0.01 \leq$ p-value < 0.05; ** = p-value < 0.01. LA: linoleic acid; ALA: alpha-linoleic acid; GLA: γ-linolenic acid. IAN: Ill anorexia nervosa; RecAN: recovered anorexia nervosa. SCD-16: stearoyl-CoA desaturase-16 (palmitoleic acid/palmitic acid); SCD-18: stearoyl-CoA desaturase-18 (oleic acid/stearic acid); D6D: delta-6-desaturase (for n-3: stearidonic acid/ALA; for n-6: GLA/LA).

3.2.4. n-6 to n-3 Fatty Acid Ratios

Ratios between well-studied n-6 and n-3 FAs were calculated to examine the relationship between n-6:n-3 ratio and AN risk (Figure 4). LA/ALA ratio was 56% lower in all AN compared to controls during fasting (p-value = 0.06) (Figure 4A) but was not statistically different in the postprandial state (p-value = 0.36, Figure 4B). Compared to controls, RecAN showed a 63% decrease in LA/ALA (p-value = 0.08) during fasting (Figure 4A) but not in the postprandial state (p-value = 0.41) (Figure 4B). In contrast, IAN showed a 267% higher ratio of postprandial LA/ALA when compared to controls (p-value = 0.018) (Figure 4B). The ratio of arachidonic acid (20:4, n-6 ARA) to EPA was marginally lower in all AN during fasting (p-value = 0.06) (Figure 4A). In RecAN, ARA/EPA ratio was significantly lower at both fasting (by 28%, p-value = 0.015) and postprandial (by 26%, p-value = 0.019) states in comparison to controls (Figure 4). ARA/DPA ratio in all AN was significantly lower than that in controls during fasting (by 32%, p-value < 0.001) but not after eating (p-value = 0.34) (Figure 4). During fasting, ARA/DPA was reduced by 37% in IAN (p-value < 0.001) and by 23% in RecAN (p-value = 0.051) when compared to controls (Figure 4A).

A number of n-6:n-3 combination ratios including ARA/DHA , (ARA + adrenic acid)/(EPA + DPA + DHA) , and (DGLA + ARA + osbond + adrenic acid)/ (EPA + DPA + DHA) were not significantly different between AN and controls in either fasting or postprandial states (Figure 4). In IAN only, fasting ARA/DHA was 29% higher than controls (p-value = 0.004; Figure 4A), while ARA + adrenic acid)/(EPA + DPA + DHA was 15% (p-value = 0.041) and 17% lower (p-value = 0.022) at fasting and postprandial timepoints in RecAN (Figure 4). Similarly, (DGLA + ARA + osbond + adrenic acid)/(EPA + DPA + DHA) was 13% lower in RecAN during fasting (p-value = 0.081) and 16% lower in postprandial state (p-value = 0.037) (Figure 4).

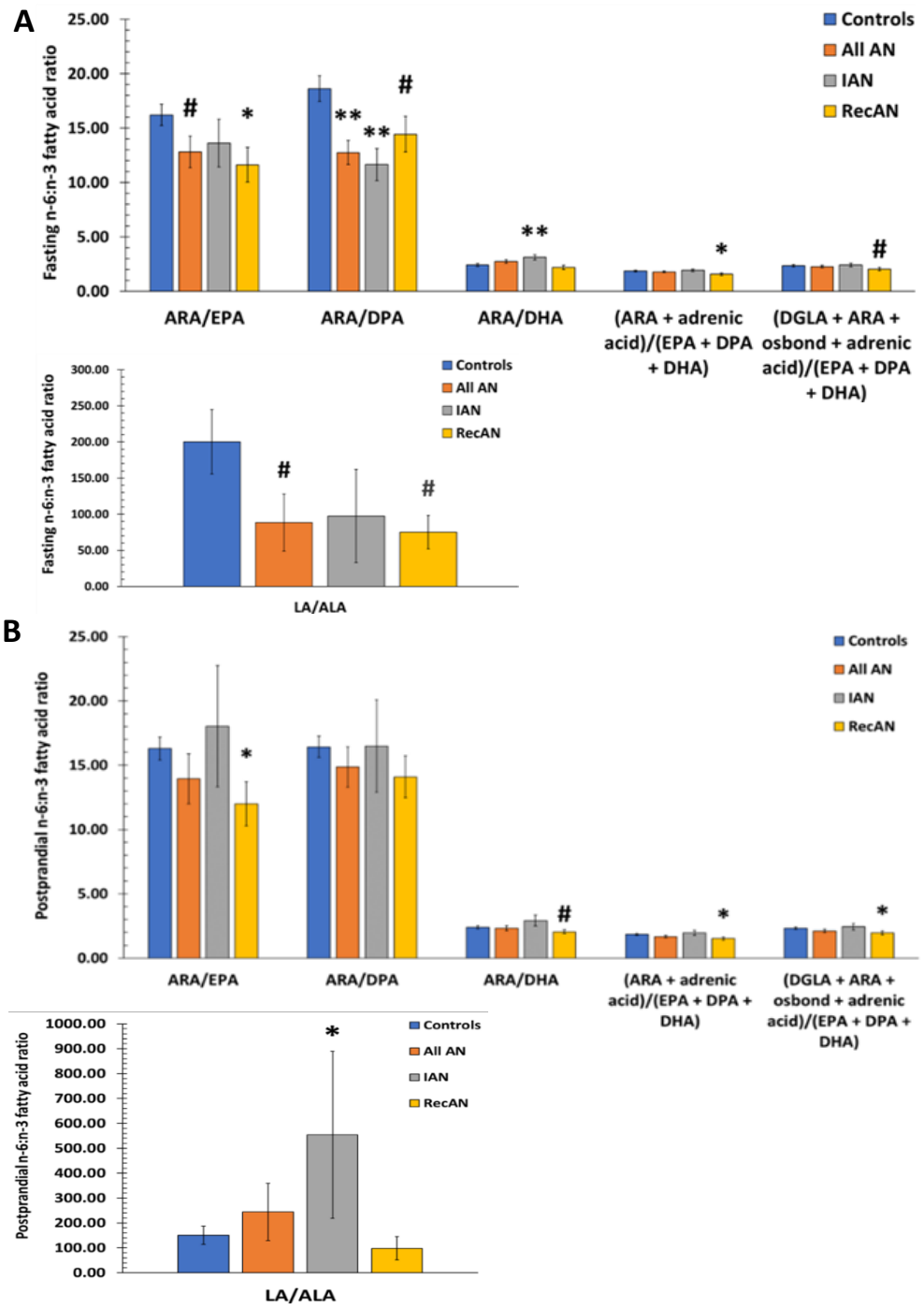


Figure 4. Fasting (**A**) and postprandial (**B**) n-6:n-3 fatty acid ratios. Entries are ratios formed by individual fatty acids. Fatty acid has unit of measurement of pmol/mL Bars and error bars represent mean and standard error of the mean. T-test was used to compare mean ratio for each individual group of AN (all AN, ill AN, recovered AN) with controls. Statistics: # = 0.05 ≤ p-value < 0.10; * = 0.01 ≤ p-value < 0.05; ** = p-value < 0.01. LA: linoleic acid; ALA: alpha-linolenic acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid. DGLA: Dihomo-γ-linolenic acid. IAN: Ill anorexia nervosa; RecAN: recovered anorexia nervosa.

3.3. Soluble Epoxide Hydrolase Expression and Activity

Albeit not statistically significant (p-values > 0.05), sEH protein expression was 19% higher in AN compared to controls at both fasting (mean ± SD: 80.05 ± 58.45 ng/mL [AN] and 67.12 ± 58.13 ng/mL [controls]; Figure 5A) and postprandial timepoints (118.28 ± 112. 03 ng/mL [AN] and 99.21 ± 35.29 ng/mL [controls]; Figure 5B). sEH activity level was 60% and 37% higher in AN than in controls during fasting (299.79 ± 218.63 pmol/mL/min [AN] and 186.93 ± 146.64 pmol/mL/min [controls]; Figure 6A) and postprandial states (408.49 ± 420.70 pmol/mL/min [AN] and 298.56 ± 117.82 pmol/mL/min [controls]; Figure 6B), respectively, although these differences did not reach statistical significance (p-values > 0.05).

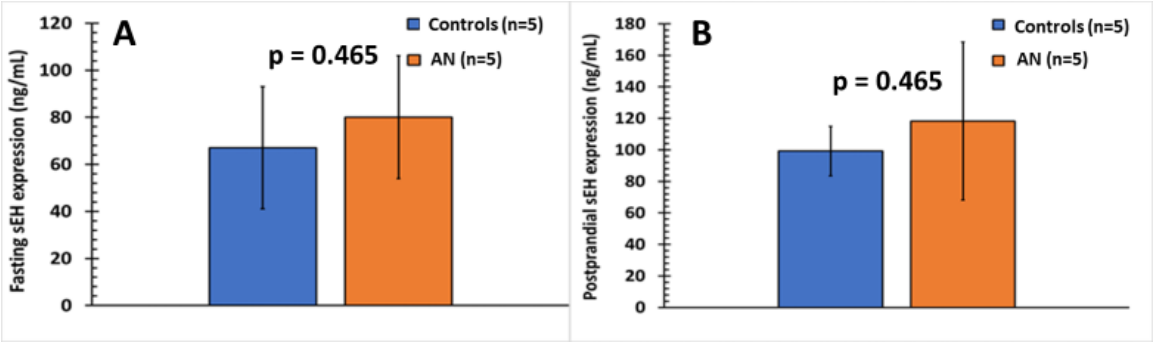


Figure 5: Fasting (**A**) and postprandial (**B**) sEH expression (ng/mL) in AN and control groups. Bars and error bars represent mean and standard error of the mean. Wilcoxon rank-sum test was used to compare mean sEH expression between all AN group and healthy control group. No statistical significance met at p-value < 0.05.

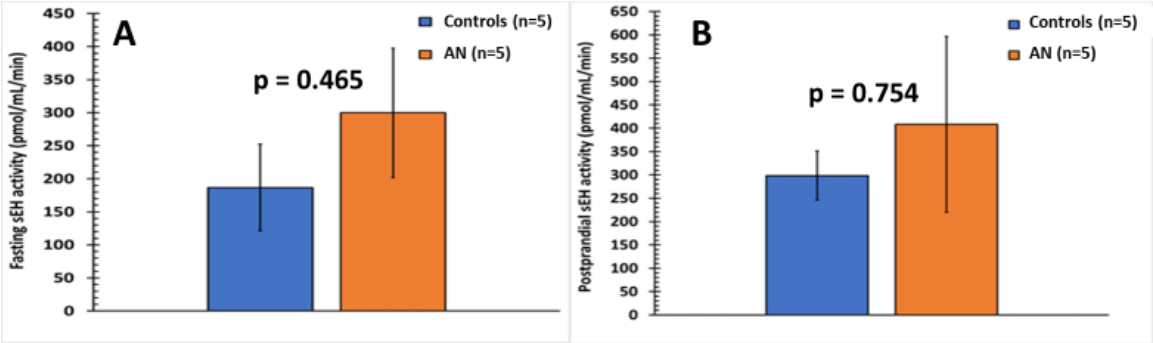


Figure 6: Fasting (**A**) and postprandial (**B**) sEH activity (pmol/mL/min). Bars and error bars represent mean and standard error of the mean. Wilcoxon rank-sum test was used to compare mean sEH expression between all AN group and healthy control group. No statistical significance met at p-value < 0.05.

3.4. Oxylipins and Their Use as Soluble Epoxide Hydrolase Activity Indices

During fasting, of the 11 epoxy fatty acids analyzed, n-6 ARA-derived 8,9-EpETrE was 52% lower in AN compared to controls (mean ± SD: 0.88 ± 0.79 nmol/L and 1.85 ± 1.38 nmol/L, respectively, p-value = 0.076) (Figure 7B). At the postprandial timepoint, n-3 ALA-derived 9,10-EpODE was more than five times higher in AN than in controls (4.06 ± 5.37 nmol/L and 0.65 ± 0.21 nmol/L, respectively; p-value = 0.047) (Figure 7C). None of the other epoxy fatty acids were significantly different between the two groups (Figure 7). However, although not significantly different between AN and controls, postprandial changes in epoxy fatty acids were generally more exaggerated in the AN group and followed an opposite direction compared to those in the control group. For example, ALA-derived 9,10-EpODE showed a significant postprandial decrease of 64% in controls (one-sample Wilcoxon test p-value = 0.043) but a non-significant postprandial increase of 270% in AN (one-sample Wilcoxon test p-value = 0.500). DHA-derived 16,17-EpDPE was lowered by 44% in controls (one-sample Wilcoxon test p-value = 0.043) but increased by 154% in AN (one-sample Wilcoxon test p-value = 0.686) after eating. EPA-derived 17,18-EpETE exhibited a postprandial decrease of 50% in controls (one-sample Wilcoxon test p-value = 0.043) but a postprandial increase of 15% in AN (one-sample Wilcoxon test p-value = 0.686). Similarly, LA-derived 9,10-EpOME was reduced by 45% in controls after eating (one-sample Wilcoxon test p-value = 0.043) while exhibiting a postprandial increase of 179% in AN (one-sample Wilcoxon test p-value = 0.892).

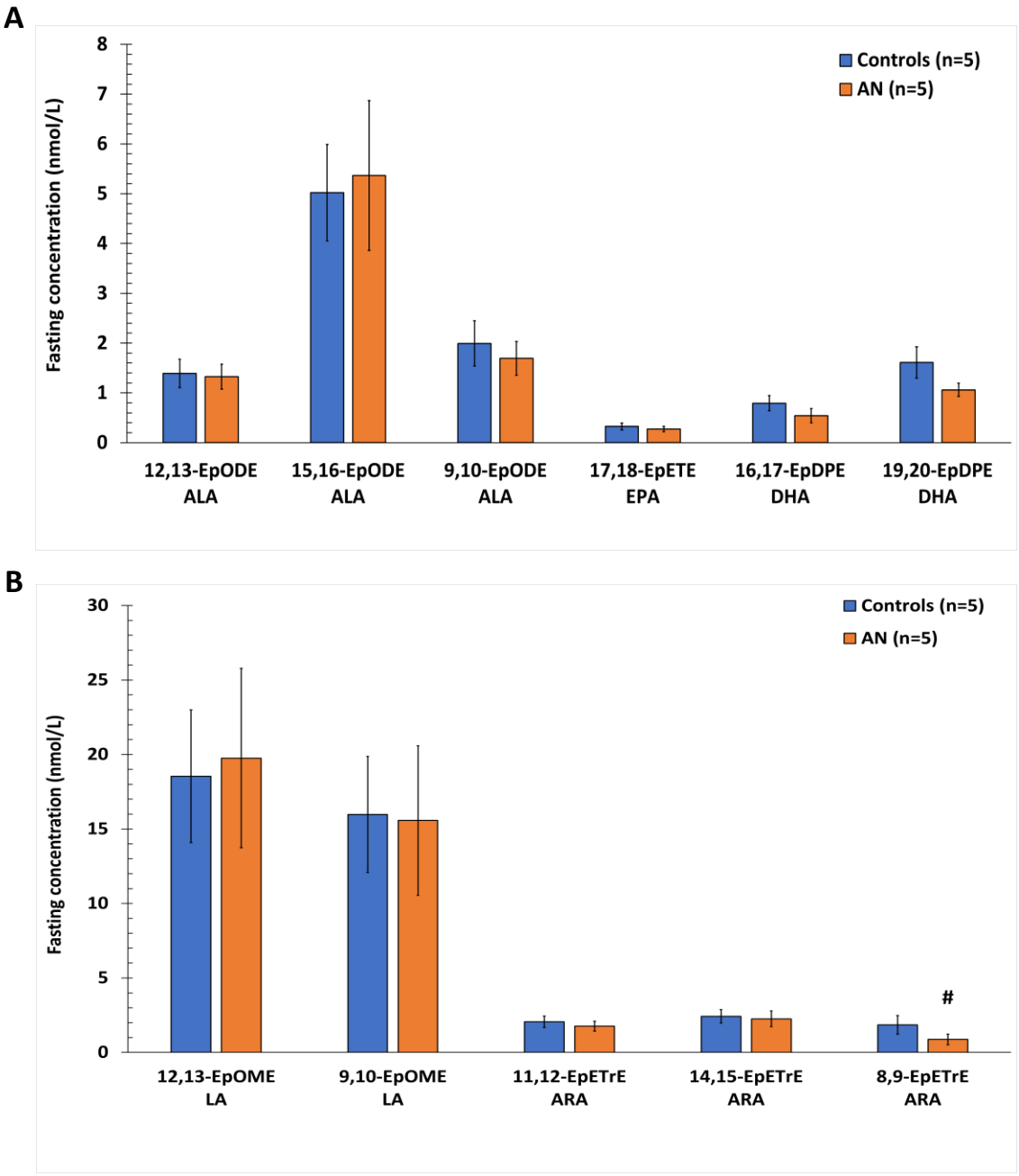
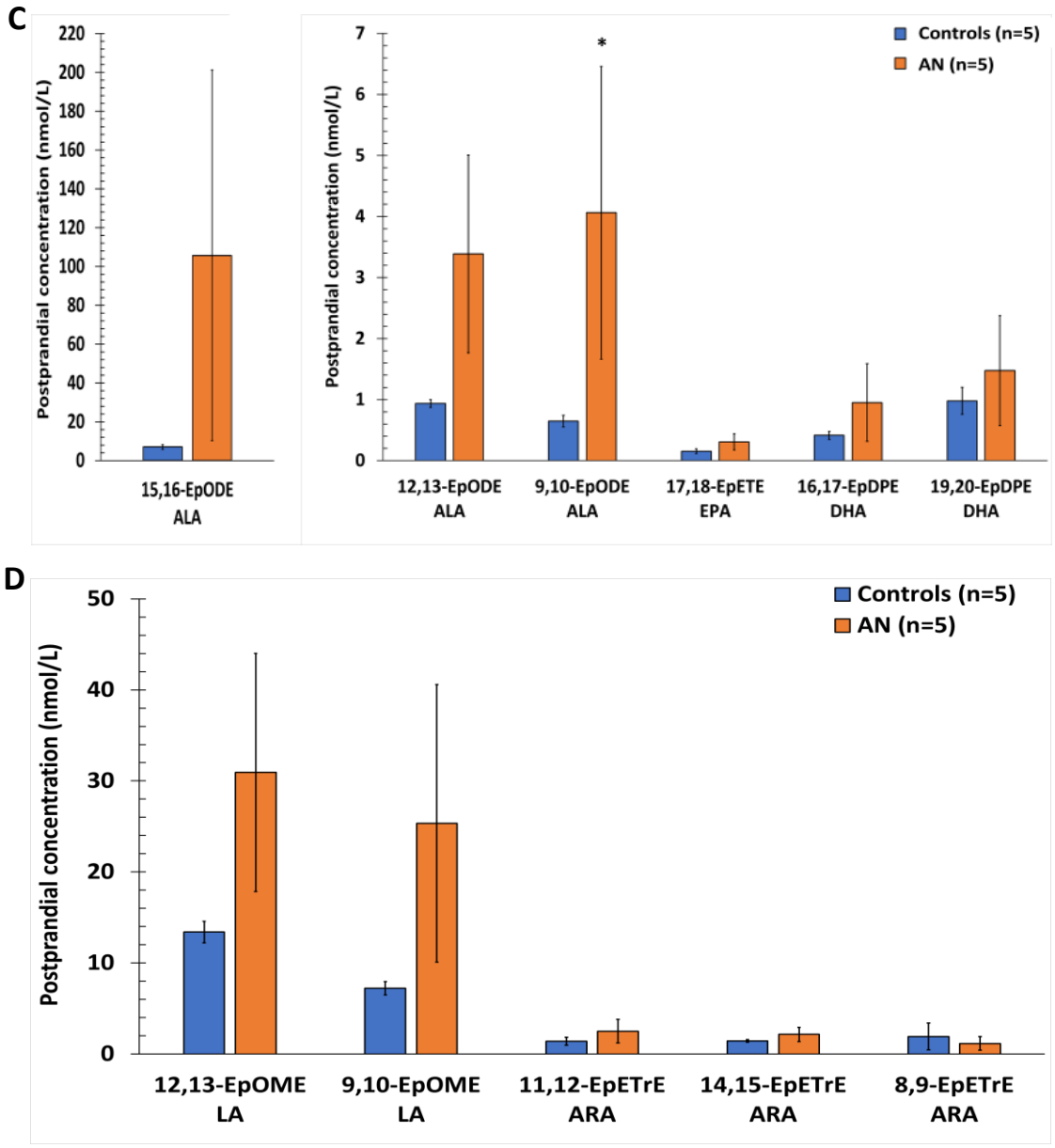


Figure 7: Fasting (**A** [n-3 FA-derived] and **B** [n-6 FA-derived]) and postprandial (**C** [n-3 FA-derived] and **D** [n-6 FA-derived]) concentrations of 11 epoxy fatty acids (nmol/L) in controls (blue) and anorexia nervosa (orange). Bars and error bars represent mean and standard error of the mean. Wilcoxon rank-sum test was used to compare mean concentrations of epoxy fatty acids between AN and control groups. Statistics: # = 0.05 ≤ p-value < 0.10; * = 0.01 ≤ p-value < 0.05; ** = p-value < 0.01.

Figure 7: continued



Among the 11 diol fatty acids examined, ALA-derived 9,10-DiHODE exhibited 191% higher fasting concentrations in AN in comparison to controls (0.58 ± 0.24 nmol/L and 0.20 ± 0.15 nmol/L, respectively; p-value = 0.028) (Figure 8A). After eating, ARA-derived 11,12-DiHETrE were 42% significantly higher in AN (0.81 ± 0.18 nmol/L and 0.57 ± 0.27 nmol/L, respectively; p-value = 0.028) (Figure 8D). A majority of the analyzed diol fatty acids showed greater postprandial increases in controls compared to AN, although none of these between-group differences were statistically significant. For

instance, ALA-derived 12,13-DiHODE and LA-derived 12,13-DiHOME and 9,10-DiHOME were increased by 130%, 125% and 199% in controls (one-sample Wilcoxon test all p-values = 0.043) but only by 75% (one-sample Wilcoxon test p-value = 0.686), 39% (one-sample Wilcoxon test p-value = 0.224), and 41% (one-sample Wilcoxon test p-value = 0.225) in AN. In contrast, AN exhibited a greater postprandial increase in ALA- derived 15,16-DiHODE (528%, one-sample Wilcoxon test p-value = 0.043) when compared to controls (105% increase, one-sample Wilcoxon test p-value = 0.080). Meanwhile, controls showed a greater postprandial reduction of 33% in ARA-derived 11,12-DiHETrE (one-sample Wilcoxon test p-value = 0.043) when compared to AN (15% decrease, one-sample Wilcoxon test p-value = 0.080).

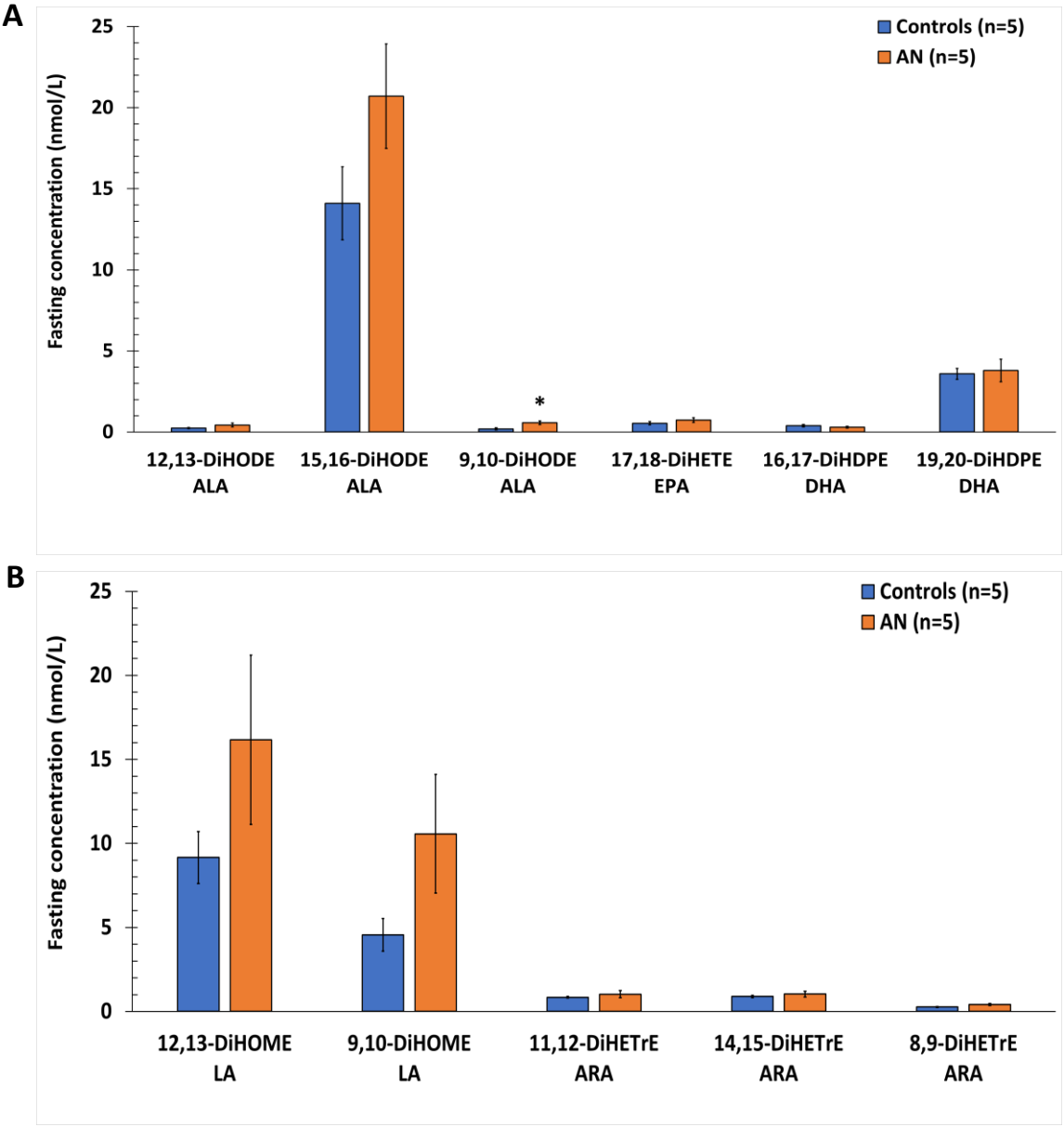
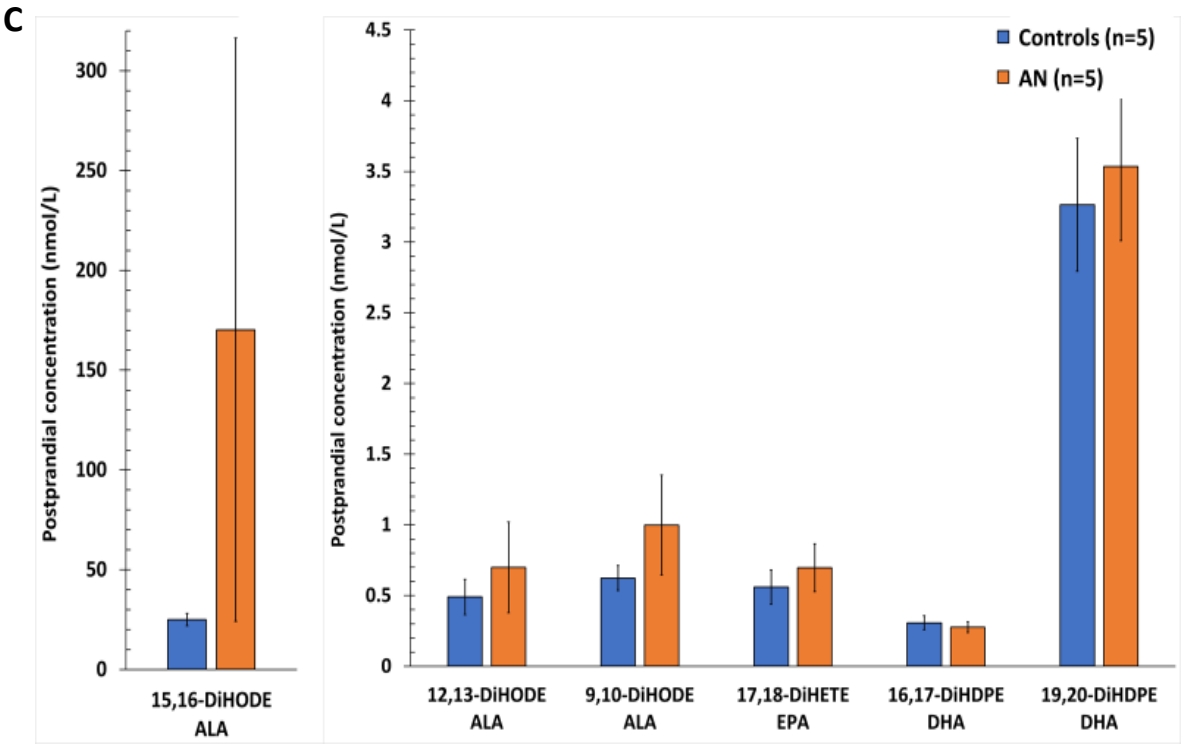
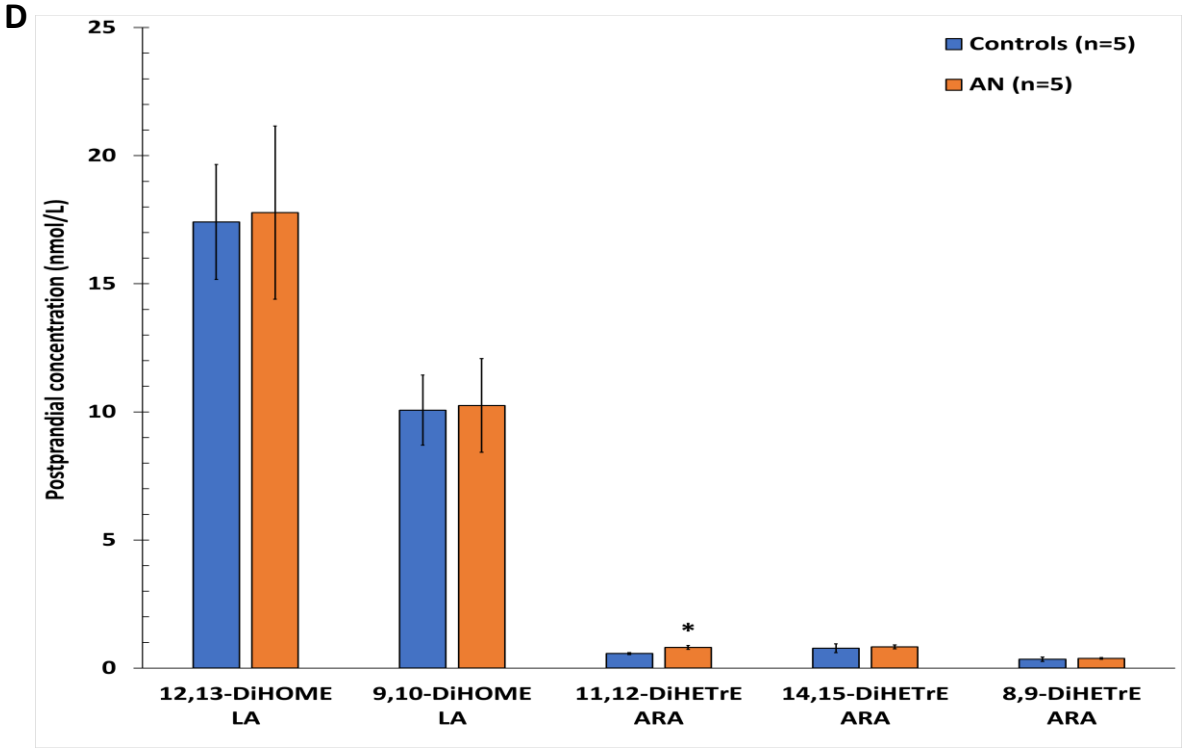


Figure 8: Fasting (**A** [n-3 FA-derived] and **B** [n-6 FA-derived]) and postprandial (**C** [n-3 FA-derived] and **D** [n-6 FA-derived]) concentrations of 11 diol fatty acids (nmol/L) in controls (blue) and anorexia nervosa (orange). Bars and error bars represent mean and standard error of the mean. Wilcoxon rank-sum test was used to compare mean concentrations of diol fatty acids between AN and control groups. Statistics: # = 0.05 ≤ p-value < 0.10; * = 0.01 ≤ p-value < 0.05; ** = p-value < 0.01.

Figure 8: continued





Under fasting conditions, of the 11 diol/epoxy fatty acid ratios, n-3 ALA-derived 9,10-DiHODE/9,10-EpODE and n-3 DHA-derived 19,20-DiHDPE/19,20-EpDPE were 305% and 47% higher

in AN (p-values = 0.047) (Figure 9A). Similarly, fasting LA-derived 9,10-DiHOME/9,10-EpOME and ARA-derived 8,9-DiHETrE/8,9-EpETrE were increased by 112% (p-value = 0.047) and 268% (p-value = 0.009) in AN (Figure 9A). After eating, 9,10-DiHODE/9,10-EpODE was 65% lower in AN (p-value = 0.028), while LA-derived 12,13-DiHOME/12,13-EpOME and 9,10-DiHOME/9,10-EpOME were 39% (p-value = 0.075) and 50% (p-value = 0.047) lower (Figure 9B).

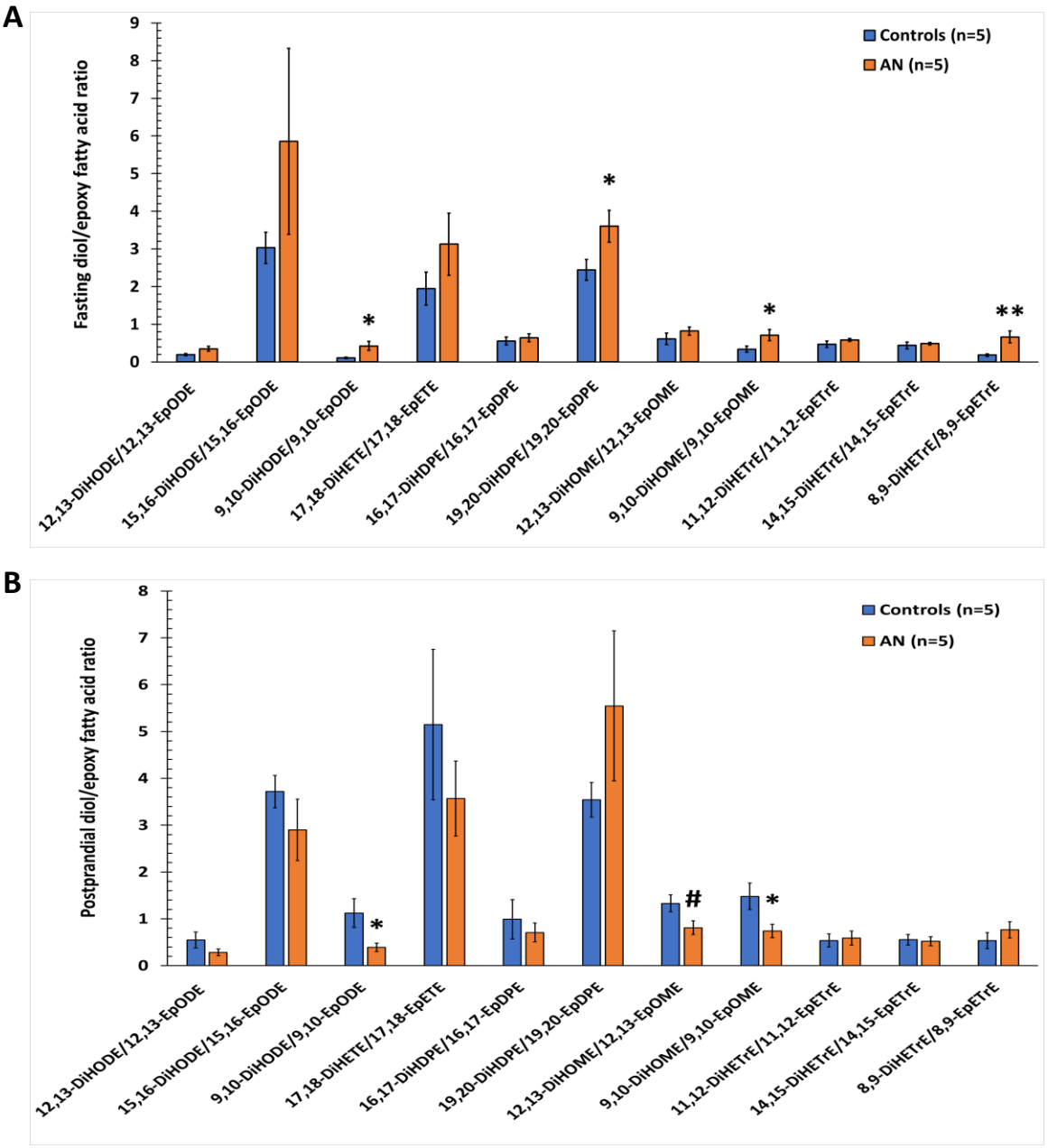


Figure 9: Fasting (**A**) and postprandial (**B**) ratios of 11 diol/epoxy fatty acid markers of in vivo sEH activity in controls (blue) and anorexia nervosa (orange). Bars and error bars represent mean and standard error of the mean. Wilcoxon rank-sum test was used to compare mean diol/epoxy fatty acid ratios between AN group and control group. Statistics: # = $0.05 \leq$ p-value < 0.10; * = $0.01 \leq$ p-value < 0.05; ** = p-value < 0.01.

3.5. Relationships between Soluble Epoxide Hydrolase and in vivo Activity Indices

Figure 10 shows the correlations between sEH expression and activity in women with AN and healthy controls at both fasting and postprandial timepoints. In both fasting (Figure 10A) and postprandial states (Figure 10B), increases in sEH expression were strongly correlated with higher levels of sEH activity in AN (fasting and postprandial: Pearson's r = 0.99, p-value = 0.001) and control group (fasting: r = 0.97, p-value = 0.006; postprandial: r = 0.92, p-value = 0.026).

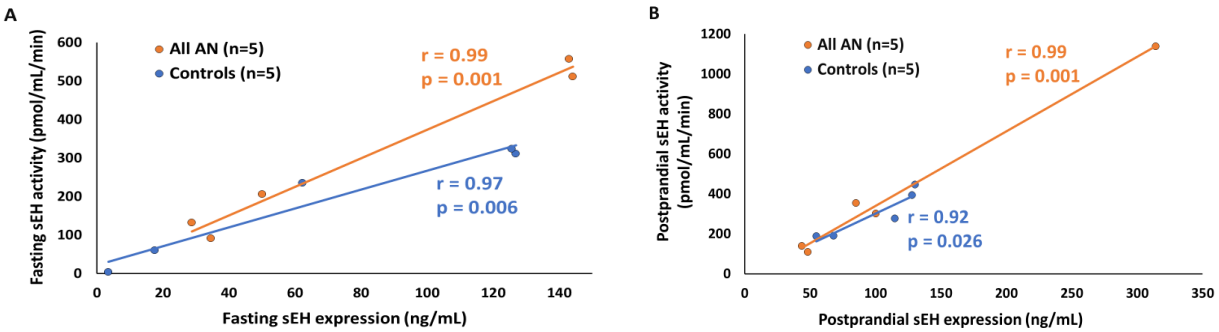


Figure 10: Scatterplot and correlations between sEH expression (ng/mL) and activity (pmol/mL/min) in anorexia nervosa (orange) and controls (blue) during fasting (**A**) and postprandial (**B**) states. Pearson correlation analyses were used to measure the strength, direction, and statistical significance of associations between sEH expression and activity. Each colored dot represents an individual's measured sEH expression (x-axis) and activity (y-axis). Solid lines represent the slopes of relationships between sEH expression and activity in AN (orange) and controls (blue). r and p indicate Pearson correlation coefficient and p-value, respectively.

Table 4 and 5 summarize the correlations between each of the 11 diol/epoxy fatty acid ratios (in vivo sEH activity markers) and sEH expression and activity at fasting (Table 4) and postprandial (Table 5) timepoints. During fasting, none of the analyzed diol/epoxy fatty acid ratios showed significant associations with sEH expression or activity in AN (Table 4). Fasting ratio of ALA-derived 9,10-DiHODE/9,10-EpODE was marginally (positively) correlated with fasting sEH expression (r = 0.84; p-value = 0.078) and sEH activity (r = 0.87; p-value = 0.056) in AN. Fasting LA-derived 12,13-DiHOME/12,13-EpOME (r = 0.81; p-value = 0.094) and ARA-derived 11,12-DiHETrE/11,12-EpETrE (r = 0.82; p-value = 0.087) also showed a trend toward positive associations with sEH expression in AN. In the control group, fasting ALA-derived 9,10-DiHODE/9,10-EpODE was significantly correlated with both sEH expression (r = 0.92; p-value = 0.027) and activity (r = 0.95; p-value = 0.015) (Table 4) but not at the postprandial timepoint (Table 5).

Marginally significant positive correlations were observed between fasting DHA-derived 19,20-DiHDPE/19,20-EpDPE and sEH activity (r = 0.81; p-value = 0.093) and between ARA-derived 8,9-DiHETrE/8,9-EpETrE and sEH expression (r = 0.85; p-value = 0.066) in controls (Table 4). In the postprandial state, ratios of LA-derived 9,10-DiHOME/9,10-EpOME, 12,13-DiHOME/12,13-EpOME, and ARA-derived 8,9-DiHETrE/8,9-EpETrE were inversely correlated with sEH expression and activity in AN (9,10-DiHOME/9,10-EpOME and sEH expression: r = −0.95. 9,10-DiHOME/9,10-EpOME and sEH activity: r = −0.93. 12,13-DiHOME/12,13-EpOME and sEH expression: r = −0.94. 12,13-DiHOME/12,13-EpOME and sEH activity: r = −0.90. 8,9-DiHETrE/8,9-EpETrE and sEH expression and activity: r = −0.97. All p-values < 0.05) (Table 5). Postprandial EPA-derived 17,18-DiHETE/17,18-EpETE was inversely correlated with sEH expression (r = −0.92; p-value = 0.025) and activity (r = −0.91; p-value = 0.032) in AN, while 19,20-DiHDPE/19,20-EpDPE was marginally correlated with sEH expression (r = −0.82; p-value = 0.092) (Table 5). At the postprandial timepoint, ARA-derived 11,12-DiHETrE/11,12-EpETrE and 14,15-DiHETrE/14,15-EpETrE also showed marginally significant inverse correlations with sEH expression (11,12-DiHETrE/11,12-EpETrE : r = −0.82; p-value = 0.087; 14,15-DiHETrE/14,15-EpETrE: r = −0.87; p-value = 0.057) and activity (11,12-DiHETrE/11,12-EpETrE : r = −0.83; p-value = 0.083; 14,15-DiHETrE/14,15-EpETrE: r = −0.85; p-value = 0.067) in AN (Table 5). The control group exhibited no significant correlations between any of the examined diol/epoxy fatty acid ratios and sEH expression or activity (Table 5).

Table 4: Pearson's correlations of n-3 (black) and n-6 (purple) FA-derived diol/epoxy fatty acid ratios with sEH protein expression and activity in AN and controls during fasting. Statistics: # = 0.05 ≤ p-value < 0.10; * = 0.01 ≤ p-value < 0.05; ** = p-value < 0.01.

Fasting diol/epoxy fatty acid (AN Group)	**sEH protein expression**		**sEH activity**	
	r	**p-value**	**r**	**p-value**
12,13-DiHODE/12,13-EpODE (ALA)	0.39	0.512	0.51	0.382
15,16-DiHODE/15,16-EpODE (ALA)	−0.24	0.693	−0.18	0.774
9,10-DiHODE/9,10-EpODE (ALA)	0.84	0.078#	0.87	0.056#
17,18-DiHETE/17,18-EpETE (EPA)	−0.59	0.292	−0.69	0.196
16,17-DiHDPE/16,17-EpDPE (DHA)	−0.19	0.760	−0.10	0.874
19,20-DiHDPE/19,20-EpDPE (DHA)	−0.61	0.270	−0.70	0.191
12,13-DiHOME/12,13-EpOME (LA)	0.78	0.118	0.81	0.094#
9,10-DiHOME/9,10-EpOME (LA)	0.72	0.168	0.75	0.141
11,12-DiHETrE/11,12-EpETrE (ARA)	0.79	0.109	0.82	0.087#
14,15-DiHETrE/14,15-EpETrE (ARA)	0.22	0.728	0.24	0.692
8,9-DiHETrE/8,9-EpETrE (ARA)	0.41	0.491	0.43	0.468
Fasting diol/epoxy fatty acid (Control Group)				
12,13-DiHODE/12,13-EpODE (ALA)	0.23	0.715	0.43	0.469
15,16-DiHODE/15,16-EpODE (ALA)	0.27	0.664	0.41	0.498
9,10-DiHODE/9,10-EpODE (ALA)	0.92	0.027*	0.95	0.015*
17,18-DiHETE/17,18-EpETE (EPA)	0.69	0.198	0.66	0.225
16,17-DiHDPE/16,17-EpDPE (DHA)	0.03	0.967	0.09	0.883
19,20-DiHDPE/19,20-EpDPE (DHA)	0.66	0.227	0.81	0.093#
12,13-DiHOME/12,13-EpOME (LA)	−0.15	0.810	−0.06	0.928
9,10-DiHOME/9,10-EpOME (LA)	0.19	0.759	0.22	0.722
11,12-DiHETrE/11,12-EpETrE (ARA)	−0.21	0.735	−0.07	0.916
14,15-DiHETrE/14,15-EpETrE (ARA)	−0.01	0.986	0.22	0.725
8,9-DiHETrE/8,9-EpETrE (ARA)	0.85	0.066#	0.79	0.110

Table 5: Pearson's correlations of n-3 (black) and n-6 (purple) FA-derived diol/epoxy fatty acid ratios with sEH protein expression and activity in AN and controls at the postprandial timepoint. Statistics: # = 0.05 ≤ p-value < 0.10; * = 0.01 ≤ p-value < 0.05; ** = p-value < 0.01.

Postprandial diol/epoxy fatty acid (AN Group)	**sEH protein expression**		**sEH activity**	
	r	**p-value**	**r**	**p-value**
12,13-DiHODE/12,13-EpODE (ALA)	−0.71	0.179	−0.71	0.180
15,16-DiHODE/15,16-EpODE (ALA)	−0.74	0.155	−0.73	0.160
9,10-DiHODE/9,10-EpODE (ALA)	−0.68	0.205	−0.66	0.225
17,18-DiHETE/17,18-EpETE (EPA)	−0.92	0.025*	−0.91	0.032*
16,17-DiHDPE/16,17-EpDPE (DHA)	−0.68	0.209	−0.68	0.203
19,20-DiHDPE/19,20-EpDPE (DHA)	−0.82	0.092#	−0.78	0.117
12,13-DiHOME/12,13-EpOME (LA)	−0.94	0.017*	−0.90	0.037*
9,10-DiHOME/9,10-EpOME (LA)	−0.95	0.013*	−0.93	0.024*
11,12-DiHETrE/11,12-EpETrE (ARA)	−0.82	0.087#	−0.83	0.083#
14,15-DiHETrE/14,15-EpETrE (ARA)	−0.87	0.057#	−0.85	0.067#
8,9-DiHETrE/8,9-EpETrE (ARA)	−0.97	0.007**	−0.97	0.006**
Postprandial diol/epoxy fatty acid (Control Group)				
12,13-DiHODE/12,13-EpODE (ALA)	0.43	0.466	0.68	0.208
15,16-DiHODE/15,16-EpODE (ALA)	0.75	0.146	0.79	0.108
9,10-DiHODE/9,10-EpODE (ALA)	0.35	0.560	0.66	0.228
17,18-DiHETE/17,18-EpETE (EPA)	0.11	0.860	0.27	0.663
16,17-DiHDPE/16,17-EpDPE (DHA)	0.35	0.569	0.60	0.286
19,20-DiHDPE/19,20-EpDPE (DHA)	−0.81	0.095#	−0.72	0.171
12,13-DiHOME/12,13-EpOME (LA)	0.23	0.708	0.58	0.309
9,10-DiHOME/9,10-EpOME (LA)	0.23	0.710	0.57	0.319
11,12-DiHETrE/11,12-EpETrE (ARA)	−0.86	0.065#	−0.82	0.086#
14,15-DiHETrE/14,15-EpETrE (ARA)	0.19	0.758	0.47	0.422
8,9-DiHETrE/8,9-EpETrE (ARA)	0.01	0.983	−0.37	0.543

In age- and BMI-adjusted multiple regression models, as fasting sEH activity increased, LA-derived 12,13-DiHOME/12,13-EpOME ratio also increased (β = 0.001; R-squared = 0.977; model p-value = 0.192) in AN (Figure 11A). In healthy controls, 12,13-DiHOME/12,13-EpOME decreased as sEH activity increased (β = −0.007; R-squared = 0.929; model p-value = 0.334) (Figure 11A). At the postprandial timepoint, when sEH activity increased, 12,13-DiHOME/12,13-EpOME was significantly decreased in

AN (β = −0.0006; R-squared = 0.9998; model p-value = 0.017) but was increased in controls (β = 0.004; R-squared = 0.875; model p-value = 0.441) (Figure 11B).

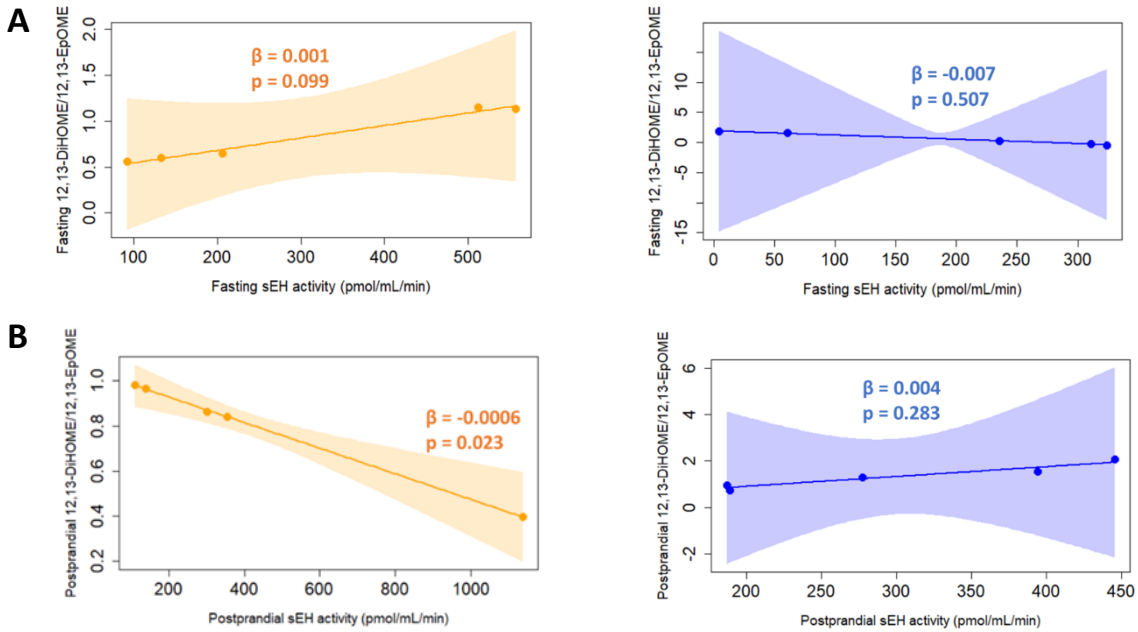


Figure 11: Age- and BMI-adjusted relationship of sEH activity with 12,13-DiHOME/12,13-EpOME in anorexia nervosa (orange) and controls (blue) during fasting (**A**) and postprandial (**B**) states. Each colored dot represents an individual residual. Solid lines indicate the slopes of relationships between sEH expression or activity on diol/epoxy fatty acid ratios when accounting for the effects of age and BMI. Shaded regions around the solid lines represent the 95% confidence intervals.

Sections 3.1-3.2, in part, are a reprint of the material as it appears in *Food-Intake Normalization of Dysregulated Fatty Acids in Women with Anorexia Nervosa*. Nguyen, Nhien; Dow, Michelle; Woodside, Blake; German, J. Bruce; Quehenberger, Oswald; Shih, Pei-an Betty, Nutrients, 2019. The thesis author was the first author of this paper.

Sections 3.3-3.5, in part, are a reprint of the material as it appears in the conference abstract *Altered Oxylipins and Soluble Epoxide Hydrolase in Women with Anorexia Nervosa*. Nguyen, Nhien; Yang, Jun; Li, Dongyang; Woodside, Blake; Hammock, Bruce; Morisseau, Christophe; Shih, Pei-an Betty, The 18th International Winter Eicosanoid Conference, 2020 (Postponed due to the 2020 COVID-19). The thesis author was the first author of this abstract.

4. Discussion

Several studies of modest sample sizes have reported fatty acid (FA) dysregulation in anorexia nervosa (AN) [26, 27, 31]. To explore the role of FA aberrancies in AN pathogenesis, we first examined the plasma concentrations of 26 well-known saturated and unsaturated FAs in women with AN and healthy controls during fasting and postprandial metabolic states. The correlative pattern of these 26 FAs in AN was distinctly different from that of controls at both fasting and postprandial timepoints (Figure 1). The differential FA signature between AN and controls, along with significant differences in concentrations of several FAs implies that lipid dysregulation plays a role in AN risk. Saturated FAs and unsaturated n-6 and n-7 FAs were more strongly correlated in controls compared to AN at both timepoints, indicating that the metabolic networks activated in AN may diminish FA correlations. Controls showed generally similar FA patterns between the fasting and postprandial timepoints, except for stronger correlations among n-6, n-7, n-9, and saturated FAs in the postprandial state. Meanwhile, AN had a notably differential FA pattern during fasting but exhibited a more similar FA pattern to that of controls after eating, implying that food intake may contribute to normalization of aberrant FA concentration in AN.

The metabolic underpinnings of AN have been demonstrated by significant genetic correlations between AN-associated variants and various metabolic features (e.g., insulin, insulin resistance, type II diabetes, and obesity) in recent GWAS consortium studies [67, 68]. A meta-analysis found that individuals with AN had elevated insulin sensitivity [69], reinforcing the link between AN and metabolic disorders. The involvement of metabolic factors, especially those related to lipid regulation in AN, suggests that studying the key players and regulators of FA synthesis and metabolism can help to clarify the biology underlying AN.

In the fasting state, three of the four AN-associated FAs belong to the n-3 polyunsaturated family (Figure 2), showing that differential FA signature in AN is mostly attributed to relative increases in n-3 FAs. The n-3 FAs are derived from dietary sources or by elongation of the essential fatty acid ALA. AN patients' dietary preference of low-calorie leafy vegetables may partially explain the disproportionately

high n-3 FAs in the circulation. ALA can be elongated to 20:3 and 22:3 FAs, which can be subsequently converted into 20:5, 22:5, and 22:6 by desaturation. Thus, metabolic compensatory mechanisms may include alterations in desaturase and elongase activities. The FAs in plasma lipoproteins are derived from either the diet or fat depots [70, 71]. Both pathways converge in the liver where FAs from either the chylomicrons or directly from the adipose tissue are repackaged and secreted as very low-density lipoprotein (VLDL) [70, 71]. Our data show a more pronounced FA profile difference in the fasting state, which suggests the involvement of adipocytes as well as evidence of metabolism regulation induced by the study challenge meal.. Adipocyte lipolysis has been found to be increased in response to starvation [72-75] and in cancer cachexia, a cancer-associated muscle wasting syndrome characterized by significant weight loss [76, 77]. Enzymes that regulate lipolysis include hormone-sensitive lipase (HSL) and adipocyte triglyceride lipase (ATGL), which hydrolyze stored triglycerides into free FAs [78, 79]. HSL gene and protein expression were significantly elevated in cancer cachexia patients compared to both weight-stable and weight-losing control cancer patients [77]. Similarly, HSL and ATGL activities were found to be higher in cancer cachexia patients than in non-cachectic cancer patients, and activity levels of both enzymes were inversely correlated with BMI in cancer/cancer cachexia [80] Genetic ablation of ATGL and HSL has also been shown to prevent or attenuate weight loss in mouse models of cancer cachexia [80]. Another enzyme regulating adipose FAs is lipoprotein lipase (LPL), the rate-limiting enzyme for FA import into adipocytes [81-83]. A lower adipose tissue LPL activity was observed in cancer cachexia models compared to controls [84]. Another study reported that adipose LPL activity was increased at lower levels of weight loss (i.e., weight loss of $\leq$ 2.5g) but decreased at greater degrees of weight loss (4.5-6 g) in cancer cachexia models [85]. Compared to an isocaloric diet, caloric restriction was linked to a lower fasting LPL activity and a greater postprandial rise in LPL activity in moderately obese women [86]. LPL mass in the serum of a female teenager with AN was found to be normal, yet this patients also exhibited other clinical phenotypes not previously associated with AN (i.e., abetalipoproteinemia and acanthocytosis, which are characterized by the absence of apolipoprotein B-containing lipoproteins in circulation and the presence of abnormal erythrocyte morphologies, respectively) [87]. Further studies are required to examine if any of these

enzymes are altered in AN, which may change the FA composition of circulating lipoproteins. Additionally, differences in pancreatic lipase activity and host factors may affect the intestinal absorption of FAs, which can alter FA concentrations in circulation. More research is needed to uncover mechanisms by which dysregulated FA signature and elevation of n-3 FAs affect AN risk, symptoms, and outcomes.

The significantly higher fasting concentrations of ALA and EPA in AN (Figure 2) are consistent with previous reports, including our previous study using an independent cohort [26, 88]. Increases in fasting lauric acid, ALA, EPA, and DPA in AN (Figure 2) are interesting, as these FAs are reported to be crucial for a healthy brain and may protect against metabolic, inflammatory, and psychiatric disorders. For example, lauric acid has been shown to promote brain health by inducing formation of ketone bodies, which serve as the brain's alternative fuel in response to impaired glucose metabolism [89]. Additionally, lauric acid exerts both antibacterial and anti-inflammatory properties as it hindered *Clostridium difficile* infection and suppressed production of inflammatory cytokines in mice [90]. Extant research has suggested that ALA may protect against coronary heart disease and stroke [91-93] and reduce autism-like phenotypes in rats [94]. EPA and DPA have been shown to be beneficial to brain health and neurodegenerative diseases [95] and are protective against metabolic syndrome: high EPA and DPA were linked to 33% and 35% reduced risk of metabolic syndrome, respectively [96]. In medical populations that experience considerable weight loss such as cancer patients, EPA has also been shown to enhance caloric intake and attenuate weight loss [97, 98]. Intake of EPA-containing supplements was associated with weight gain and higher lean body mass in pancreatic cancer patients with cachexia [99]. In a meta-analysis, adjunctive treatment with n-3 supplements containing ALA, EPA, or EPA and DHA have ameliorated depressive symptoms in bipolar disorder [100]. In schizophrenia, while the use of EPA-containing supplement in combination with antipsychotics did not confer significant benefit compared to standard antipsychotic monotherapy [101], n-3 supplementation has been shown to provide neuroprotective benefits to halt the development of psychotic disorders in ultra-high-risk individuals [102]. Similarly, research suggests beneficial effects of DPA in brain health and neurodegenerative diseases [95]. The generally beneficial effects of these FAs in other medical

or psychiatric disorders have rendered their relative elevation in AN compared to healthy controls paradoxical.

In an earlier study 28 patients with eating disorders who took n-3 FA supplements during treatment exhibited improvements in body weight [88]. In two small treatment studies, 8 AN patients who received EPA supplementation as an adjunct therapy showed an increase in weight gain [103, 104]. These findings contradict the inverse correlations between BMI and ALA in AN in another study [105], and the inverse correlations of BMI with ALA and DPA in AN in our study (Table 2). Here, we replicated the results of increased n-3 FAs from our 2016 report [26] using a stricter, experimentally-controlled study design. At the postprandial timepoint, dysregulated FAs in AN have largely normalized with the exception of ALA (Figure 2), suggesting that a single meal may "activate" or "correct" lipid metabolism in AN and normalize the differential FA signature observed in the fasting state. Together, these data demonstrate that clinical improvements observed in earlier eating disorder studies may be a result of overall increases in total calories during treatment. More studies are required to clarify the efficacy of n-3 supplement in AN treatment, and the mechanism by which n-3 dysregulation affects AN.

Previous research has documented higher n-6:n-3 FA ratios in psychiatric disorders [106-108] and in comorbid depression among girls with eating disorders [109]. In contrast, a recent meta-analysis found that patients with eating disorders have a reduced ratio of n-6:n-3 FAs compared to healthy controls [88], which is consistent with our previous [26] and current findings. In this study, all AN patients showed lower fasting ratios of LA/ALA, ARA/EPA, and ARA/DPA (Figure 4A) compared to controls. A majority of the n-6: n-3 FA ratios also showed decreases in both ill AN (IAN) and recovered AN (RecAN) compared to controls (Figure 4). Decreased n-6:n-3 ratios are partially driven by the abnormally high concentrations of n-3 FAs in our study, suggesting that the health benefits of n-3 FAs reported in other conditions [110] may not be equally beneficial for individuals with eating disorders.

In our combined correlation analysis (which included both AN and control groups), four AN-associated FAs were significantly correlated with lower BMI (Table 2), implying that these FAs may protect

against obesity and contribute to the low body weight that individuals with AN exhibit. These results are consistent with the literature showing higher ALA concentration correlating with a smaller increase in BMI in children 5–12 years old [111]. Yet, another study found no association between lauric acid consumption and BMI in adults [112]. Depression and anxiety are common mental health issues in the general population that are found to be intensified in individuals with AN. Depression and anxiety levels were positively correlated with ALA, EPA, and DPA in all subjects, indicating that these FAs and/or the metabolic pathways that preferentially utilize them may affect depression and anxiety risk. While one study reported that higher ALA and EPA concentrations were associated with fewer depression and suicide attempts [113], a meta-analysis found that eating disorder patients supplemented with n-3 FAs (mainly EPA and/or DHA) did not show improvements in mood symptoms [88]. Conflicting results may be attributed to heterogeneity in study subject characteristics and sample sizes, differences in assessment methodology and instruments, and discrepancies in the dosage and administration period of n-3 supplementation across different studies.

Correlation analyses stratified by the presence or absence of AN suggest that the inverse association with BMI was driven by the AN group. AN exhibited a significant inverse correlation of BMI with fasting ALA and DPA, while no significant BMI correlation was observed in the control group (Table 2). Both non-stratified and stratified data illustrated differences in correlations between fasting and postprandial timepoints (Table 2, 3), implicating that food intake modulated the relationships between FA concentrations and BMI, depression, and anxiety. After eating, ALA and EPA levels were significantly associated with higher postprandial anxiety in controls but not in women with AN (Table 3). The difference observed with anxiety can be explained by a significantly higher baseline (fasting) anxiety and the resulting higher relative decrease of anxiety after eating in AN (Table 1).

Saturated and unsaturated FAs can undergo desaturation and elongation to form longer-chain and more unsaturated FAs. FA desaturation and elongation are catalyzed by desaturases and elongases, which have been reported in various psychiatric disorders, including schizophrenia, bipolar disorder, and eating disorders [29, 114-117]. Product/precursor ratios of FAs were used as proxy markers of in vivo desaturase

and elongases activities in this study. Proxy markers of SCD-18 and D6D were lower in all AN patients compared to controls at fasting but not postprandial state (Figure 3), suggesting that FA desaturase activities contribute to the differential FA signature in AN. SCD-18 catalyzes the biosynthesis of the monounsaturated 18:1 oleic acid from the saturated 18:0 stearic acid, thus playing important roles in de novo lipogenesis as well as energy/lipid storage and metabolism [118]. The reduction in SCD-18 activity index among AN is consistent with previous findings that a lower SCD-18 (as well as SCD-16) index was correlated with lower body fat and BMI in older individuals [119], and that downregulation of SCD-1 (the collective name referring to both SCD-16 and SCD-18) may hinder adiposity in leptin-deficient obese mice [120]. However, a few studies showed that eating disorders patients had significantly elevated SCD-16 and SCD-18 indices compared to healthy controls [29, 30, 116]. Altogether, these observations imply that the effects of SCD on body weight may be disease-specific and population-dependent. D6D plays an important role in FA synthesis by converting dietary ALA and LA to FAs with more double bonds [121]. A lower D6D activity index was significantly correlated with a higher intake of butter/butter-oil mixture and vegetable-based margarine (60%-80% fat) in children, suggesting that D6D activity may be hindered by increased PUFA (e.g., ALA and LA) consumption [122]. D6D activity index was elevated in men with abdominal obesity and metabolic syndrome [123] and in postmortem brain samples of schizophrenia [117] and bipolar disorder patients [115] compared to controls. In contrast, decreased D6D activity was reported in girls with eating disorders compared to controls [29], which is in agreement with our data. D5D activity index was found to be higher in teenage girls with eating disorders in one study [116] but lower in adult women with AN compared to controls in another study [30]. These mixed results demonstrate a need to further research the roles of desaturase activities in AN risk and metabolic dysregulation.

sEH has been found to be involved in various physiological processes, including inflammation [38, 39]. Animal studies have shown that sEH inhibition can reduce inflammation [124-128] and confer beneficial effects in the treatment of inflammatory pathologies [128-130]. sEH inhibitors have been reported to suppress inflammatory cytokine release and thwart the development of depressive behaviors

[125] in inflammation-induced and social defeat stress mouse models of depression, while attenuating behavioral impairments in schizophrenia models [131]. Additionally, sEH protein level was higher in the brain of mice with depression-like behaviors and in postmortem brains of patients with depression, schizophrenia, and bipolar disorder compared to postmortem brains of healthy individuals [125]. These findings are in agreement with increased circulating sEH activity and expression in AN at both fasting and postprandial timepoints.

Oxylipins were quantified to assess the relationship between sEH and seasonal depression disorder [132]. Three diol fatty acids derived from LA and DHA were increased while one ARA-derived diol and one EPA-derived epoxy fatty acid were decreased in the depressed state of patients in the winter season [132]. These results suggest an association between high sEH activity and more severe depressive episodes. The diol/epoxy fatty acid ratios used as proxy markers of sEH activity were increased in AN compared to controls [26], confirming the likely modulatory role sEH plays in AN risk and comorbidities such as depression and anxiety.

The lower postprandial ALA- and LA-derived diol/epoxy fatty acid ratios found in AN can be explained by the increases in the epoxy fatty acids. LA-derived 9,10-EpOME and 12,13-EpOME exhibited remarkably large postprandial increases of 179% (p-value = 0.893) and 131% (p-value = 0.500) in AN (data not shown). In controls, these epoxy fatty acids decreased by 45% (p-value = 0.043) and 6% (p-value = 0.686) after the meal. Similarly, ALA-derived 9,10-EpODE was increased by 270% (p-value = 0.500) in AN but reduced by 64% (p-value = 0.043) in controls after eating. In the postprandial state, significantly higher concentrations of 9,10-EpODE in AN compared to controls (magnitude: 529%; p-value = 0.047; Figure 7C) may account for the significantly lower postprandial ratio of 9,10-DiHODE/9,10-EpODE. Higher levels of these epoxy fatty acids in AN might be attributed to increased concentrations of their precursor FAs in AN (postprandial LA: 9% higher in AN than controls; FDR-adjusted p-value > 0.05; postprandial ALA: 58% higher in AN; FDR-adjusted p-value = 0.046) (Figure 2B). Our postprandial data showed significant changes in oxylipin levels and in vivo sEH activity after eating in AN.

The precursor FAs are not the only factors influencing concentrations of oxylipins. Epoxy and diol fatty acids are also affected by other synthesis or degradation mechanisms such as phospholipid remodeling [133], β-oxidation [52], and ω-hydroxylation [134]. The diol/epoxy fatty acid ratios may therefore be influenced by factors other than sEH. In this study, a majority of the examined oxylipin ratios showed a similar pattern suggesting that sEH had a consistent impact on these oxylipins. In the absence of direct sEH measurement, oxylipin ratios can therefore be used as proxy markers of in vivo sEH activity. Studying in vivo sEH activity in parallel with its gene expression and activity would enable a more comprehensive understanding of sEH and its interactions with complex networks in the human body.

As expected, sEH expression and activity exhibited strong positive correlations in both fasting and postprandial states. However, the examined diol/epoxy fatty acid ratios exhibited significant positive correlations with sEH during fasting but significant inverse correlations with sEH at the postprandial timepoint, indicating that consumption of the challenge meal may modulate the relationship between sEH and oxylipins differentially in AN. In AN, more pronounced negative correlations were observed especially in the postprandial state. After eating, all five epoxy fatty acids (i.e., 9,10-EpODE, 17,18-EpETE, 8,9-EpETrE, 12,13-EpOME, and 9,10-EpOME) constituting the five diol/epoxy FA ratios showed significant positive associations with both sEH expression and activity in AN (range of r: 0.96 to 0.99; p-values < 0.05). In contrast, none but one (9,10-DiHODE) of the corresponding diol fatty acids showed significant associations with sEH expression and activity in AN in the postprandial state. These data suggest that significant postprandial epoxy fatty acids rise in AN is absent in controls, The sEH catalytic actions on epoxy fatty acids may not have been initiated or completed in time after eating for AN, resulting in negative correlations between postprandial oxylipin ratios and sEH.

The relationships between sEH activity and proxy markers of in vivo activity remain after adjustment for age and BMI. When the effects of age and BMI were accounted for, the directions of relationships between sEH activity and LA-derived 12,13-DiHOME/12,13-EpOME were consistent with the unadjusted analyses. Fasting sEH activity and 12,13-DiHOME/12,13-EpOME exhibited a positive relationship in AN but a negative relationship in controls in multivariate linear model (Figure 11A). After

eating, sEH activity showed a significant inverse association with 12,13-DiHOME/12,13-EpOME in AN but a non-significant positive relationship in controls (Figure 11B). The unexpected significant inverse relationships between sEH activity and its in vivo activity index in AN may be due to a delayed postprandial sEH action (as previously explained). Non-sEH-associated factors and pathways may also affect concentrations of the individual epoxy and diol fatty acids, leading to a negative association between sEH and its in vivo activity.

This study has several strengths. A major strength of this study included validation by replication of our earlier published results [26]. In the present study, we have successfully replicated our prior study findings of increased n-3 FAs and sEH in vivo activity in AN compared to controls. Also, we have selected well-matched AN patients and controls for this work (Table 1) to ensure that observed differences between the two groups were not due to common confounding effects. Additionally, we were able to tightly control the study protocol to capture both fasting and postprandial measurements to assess changes in FAs and their metabolites following a controlled food intake. Overall, food consumption normalized most of the relative differences in FA concentrations between AN patients and controls. Moreover, food intake altered relationships between FA concentrations and AN phenotypes (BMI, depression, and anxiety levels).This study applied multiple comparison correction and enabled visualization of relationships amongst all FAs detected using mass-spectrometry lipidomics, thus providing a comprehensive picture of FA signature in AN. We also directly assayed sEH activity and expression levels and examined them together with proxy in vivo sEH activity formed by oxylipin ratios. Lastly, a standardized sandwich was distributed to all participants in our study to keep the amounts of Calories and fats consistent and to eliminate the possibility that observed differences between AN patients and controls in the postprandial state resulted from variations in the meal.

The present study was limited by the use of FA ratios as proxy markers of desaturase and elongase activities. Future studies can explore these enzyme activities using more direct measurements. While it would have been ideal if the AN and control groups were similar in sample sizes at both timepoints, the

postprandial sample size in the AN group was smaller due to patients' refusal to eat the meal. The data presented on sEH and oxylipin was derived from a subset of five AN and five control women. Thus, it is necessary to replicate these results in larger sample sizes to ensure the validity of these exploratory data. Since inflammatory cytokines were not measured in this study, we were not able to fully determine how sEH modulates inflammation. Lastly, since cause-and-effect relationships could not be determined in the present study. it is unclear whether FA and oxylipin aberrations precede or follow/develop from AN onset.

In the future, our lab will focus on investigating sEH and inflammatory cytokines in study participants. We will also explore the effect of dietary intake on multiple postprandial timepoints, especially because individuals with AN have been shown to exhibit delayed gastric emptying [135-140] and intestinal transit [141, 142]. The complex dynamics of gastrointestinal transit, nutrient uptake, and postprandial metabolism are highly variable across the human population. Discouragingly, relatively few studies have been conducted to describe the full range of human diversity. The variation in gastrointestinal dynamics is attributable in part to genetics [143], diet [144], infection [145], and the microbiome [146]. We anticipate that assessment of multiple timepoints in our study population will help to eliminate possible biases in the current study design.

In this study, data obtained from blood samples of study participants have suggested dysregulated metabolism of FA and their metabolites in AN compared to controls. However, the extent to which blood levels of FAs, oxylipins, and sEH expression and activity reflect those in the brain remains unexplored. Discrepancies in plasma and cerebral cortex oxylipin levels have been reported in rats fed different amounts of dietary LA [133]. In addition, plasma and brain FA profiles exhibited no significant correlations in individuals with mild cognitive impairment or Alzheimer's disease, while only DHA showed significant concordance between plasma and brain tissue in people without cognitive impairment [147]. Since AN is a psychiatric disorder accompanied by alterations in brain structure and circuitry [148], more studies are needed to determine whether alterations in FAs, oxylipins, and sEH in the circulation are present also in the brain.

FA signatures at both fasting and postprandial states were visually different in AN compared to controls, espousing the metabolic dysfunctions known in AN. Our data showed that eating a high-fat sandwich appears to normalize these dysregulations. Saturated and n-9, n-7, n-6, and n-3 FAs are synthesized by ostensibly all higher organisms including humans. The implications of these FAs in whole body metabolism, especially fuel regulation, were first demonstrated by Cao et al. [149]. Sources of these FAs include the diet, de novo synthesis, or mobilization from adipose or tissue stores. While it is not possible to determine which factor is most important in driving the changes observed in AN without exhaustive isotope enrichment studies, the correlations and associations identified here enable us to enrich or deplete these FAs using dietary, pharmacologic or even microbial protocols to more precisely study mechanisms of action and improve therapeutic efficacy. Future studies with larger sample sizes are needed to confirm these findings and to investigate how food intake affects FAs and their metabolites under the influence of sEH actions to influence AN risk and outcomes. Answers to these research questions will help to clarify the biological underpinnings of AN and allow for the development of improved prevention and therapeutic strategies.

The Discussion, in part, is a reprint of the material as it appears in *Food-Intake Normalization of Dysregulated Fatty Acids in Women with Anorexia Nervosa*. Nguyen, Nhien; Dow, Michelle; Woodside, Blake; German, J. Bruce; Quehenberger, Oswald; Shih, Pei-an Betty, Nutrients, 2019. The thesis author was the first author of this paper.

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