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Obesity is positively associated with arachidonic acid-derived 5- and 11-hydroxyeicosatetraenoic acid (HETE)

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ABSTRACT

Background. Oxylipids are oxygenated polyunsaturated fatty acid (PUFA) metabolites that are responsible for the onset and resolution of the inflammatory response. Enzymatic oxygenation through the lipoxygenase (LOX) or cytochrome P450 (CYP) pathways can form oxylipids that have either proinflammatory or proresolving functions depending on the type of PUFA substrate and degree of metabolism. The objective of this study was to determine how PUFA substrates and their corresponding oxylipids are associated with obesity.

Methods. Plasma non-esterified FA and oxylipids were isolated from 123 Caucasian males using solid phase extraction and quantified using high performance liquid chromatography–tandem mass spectrometry. Statistical analyses included linear regressions and polytomous logistic regressions, and the responses were body mass index (BMI) and waist circumference (WC), and serum leptin, total adiponectin, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-peptide. Models were adjusted for age and smoking, and *p*-values were corrected for false discovery per Benjamini–Hochberg and Bonferroni.

Results. We report that BMI, WC, and several serum cytokines were highly associated arachidonic acid (ARA)-derived hydroxyeicosatetraenoic acids (HETEs), and vicinal diols (i.e., alcohols on adjacent carbon atoms) derived from several PUFAs. There was a significant linear relationship between BMI, WC, and serum leptin, and ARA-derived 5-, 11-, and 15-HETE. Specifically, BMI and WC were positively associated with proinflammatory 5- and 11-hydroxyeicosatetraenoic acid (HETE), even after normalization to ARA concentrations and false discovery p-value correction. Individuals with 5-HETE concentrations >5.01 nmol/L or 11-HETE concentrations and >0.89 nmol/L were over 5 times more likely to be obese compared to those with ≤1.86 nmol/L and ≤0.39 nmol/L, respectively.

Abbreviations: AEA, arachidonoyl ethanolamide; AG, arachidonoyl glycerol; ALA, alpha-linolenic acid; ARA, arachidonic acid; BHT, butylated hydroxytoluene; BMI, body mass index; COX, cyclooxygenase; CYP, cytochrome P450 enzymes; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamide; DHET, dihydroxyeicosatrienoic acid; DiHETE, dihydroxyeicosatetraenoic acid; DiHOME, dihydroxyoctadecadienoic acid; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; EpOME, epoxyoctadecenoic acid; FA, fatty acid; HDPA, dihydroxydocosapentaenoic acid; HETE, hydroxyeicsaotetraenoic acid; HETrE, hydroxyeicosatrienoic acid; HFD, high-fat diet; HODE, hydroxyoctadecadienoic acid; HOTrE, hydroxyoctadecatrienoic acid; HPLC, high performance liquid chromatography; oxoODE, keto octadecadienoic acid; LA, linoleic acid; LOX, lipoxygenase; LTB4, leukotriene B4; MRM, multiple reaction monitoring; MS/MS, tandem mass spectrometry; PGE2, prostaglandin E2; PUFA, polyunsaturated fatty acid; sEH, soluble epoxide hydrolase; TBX2, thromboxane B2; WC, waist circumference; ω-3, omega-3; ω-6, omega-6.

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Vicinal diols from linoleic, eicosapentaenoic, and docosahexaenoic acid were inversely associated with obesity. Across all statistical tests, vicinal diols were inversely associated with obesity whether normalized to parent PUFA concentrations or normalized to precursor epoxides. Interestingly, the proinflammatory cytokines IL-6 and TNF-α were not associated with any oxylipids. Since 5-HETE is a 5LOX product, 11-HETE is marker of lipid peroxidation, and vicinal diols are formed through soluble epoxide hydrolase (sEH) metabolism of CYP epoxygenated PUFAs, therefore, these results indicate that obesity is likely associated with altered metabolism with distinct oxygenating pathways. Taken together, our results indicate that obesity is associated with specific oxylipids indicative of altered PUFA metabolism through several pathways (i.e., LOX, reactive oxygen species, and sEH and CYP epoxygenase), rather than attributed solely to altered dietary PUFA intake.

1. Introduction

Chronic low-grade inflammation is causally linked to the pathogenesis of obesity-associated metabolic diseases, such as insulin resistance, type-2 diabetes, cardiovascular disease and non-alcoholic fatty liver disease [1–3]. The caloric overload in obesity results in lipid accumulation in adipose tissue that triggers circulating monocyte recruitment into lipid-laden tissue [1]. Macrophage infiltration and accumulation in lipid-laden adipose tissue is a defining feature of the chronic, low-grade inflammatory state in obesity [3]. In fact, each excess kilogram of human fat results in the accumulation of an estimated 20–30 million macrophages [4]. One primary mechanism through which macrophages regulate inflammation is through the production of potent bioactive lipid mediators referred to as oxylipids. Oxylipids are oxygenated fatty acid (FA) metabolites that are responsible for the onset and resolution of the inflammatory response [5,6]. When the resolution of inflammation fails, it perpetuates a pro-oxidant environment and results in a positive feedback loop leading to the destruction of tissues [2,7–9].

Oxylipids are potent bioactive lipid mediators that have essential roles in normal physiology and function. Oxylipids formed from polyunsaturated fatty acids (PUFAs) are important mediators of PUFA effects in the body. PUFAs within membrane phospholipids serve as substrates for the biosynthesis of oxylipids through either enzymatic or non-enzymatic pathways [10]. Obesity is associated with Westernized dietary intake patterns [11], which alters phospholipid FA composition of both plasma and cellular membranes [12]. There is enzymatic competition for elongation, desaturation, and oxygenation of several structurally similar omega-6 (ω-6) and omega-3 (ω-3) PUFAs. Accumulations of one PUFA family (i.e., ω-3) will offset production of the other (i.e., ω-6) and can impact oxylipid production. For instance, increased plasma ω-3 are associated with higher concentrations of plasma ω-3 oxylipids and a concomitant reduction in plasma ω-6 [13].Oxylipids are endogenously synthesized by the enzymes cytochrome P450 (CYP) enzymes [14], cyclooxygenase (COX) [15], lipoxygenase (LOX) [16], and non-enzymatic pathways such as reactive oxygen species (ROS), especially during oxidative stress. PUFAs such as linoleic (LA), alpha-linolenic (ALA), arachidonic acid (ARA), dihomo-γ-linolenic acid (DGLA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are substrates for these oxygenating enzymes and non-enzymatic reactions.

Obesity is associated with pro-oxidative stress and increased ROS [17]. ROS are formed as a normal end products of cellular metabolism and by phagocytic cells, such as macrophages during inflammation. CYP metabolism of PUFAs form abundant classes of oxylipids such as PUFA epoxides (i.e., formed by CYP2J2, CYP2C8, and CYP2C9) [18] and omega-hydroxy PUFA (i.e., formed by CYP4F, CYP4A, and CYP2U) [18]. There are several CYP epoxygenases with preferential affinity to PUFA substrates. In particular, the epoxygenase CYP2J2 family is highly expressed in tissues affected by obesity-associated pathologies such as liver and skeletal muscle [19], adipose tissue [20], pancreas [21], and cells such as macrophages and monocytes [22,23]. In general, PUFA epoxides are described as more “anti-inflammatory like” [24]. For instance, CYP2J2 activity and PUFA epoxides (i.e., ARA-derived epoxides) inhibit NF-κB, thus, reducing inflammatory responses [25]. However, some epoxides such as LA-derived epoxides produced by cells such as macrophages are commonly referred to as leukotoxins [26–28]. PUFA epoxides are further metabolized to form vicinal diols (i.e., alcohols on adjacent carbon atoms) by the enzyme soluble epoxide hydrolase (sEH), and vicinal diols are described as more “inflammatory like” [18].

Recently, we reported that pooled obese plasma had lower concentrations of vicinal diols (the end products from sEH metabolism of PUFA epoxides), and higher concentrations of oxylipids associated with proinflammatory function including: 5-hydroxyeicsaotetraenoic acid (HETE) (5LOX product), 11-HETE (non-enzymatic product), 15-HETE (15LOX product), and 9,10- and 12,13-epoxyoctadecenoic acid (12,13-EpOME; CYP epoxygenase products) compared to pooled lean plasma [29]. Pooling is a useful method to assess preliminary differences, however, no statistical assessment of variation in the data can be performed. In our current study, plasma oxylipids were quantified using an expanded profile of 62 plasma oxylipids to determine oxylipid classes and concentrations significantly associated with body mass index (BMI), waist circumference (WC), and serum leptin, total adiponectin, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-peptide in 123 males. The proinflammatory serum cytokines IL-6 and TNF-α were not associated with plasma oxylipids. After false discovery correction, vicinal diols were inversely associated with BMI, WC, leptin, and C-peptide, and 5- and 11-HETE were positively associated with BMI, WC, and serum leptin. Associations between several responses, and vicinal diols and HETEs (i.e., 5- and 11-HETE)

were significant even after normalization to plasma concentrations of parent PUFAs.

2. Materials and Methods

2.1. Study Population and Serum Cytokine Quantification

Data are further described elsewhere [29–34]. In brief, healthy and asymptomatic male subjects (n = 123, >96% Caucasian) 48 to 65 years of age were enrolled between 2009 and 2011 in a cross-sectional study. Data comprise clinical metadata on individuals' co-morbidities, family history, and use of medications. Individuals with the following medical conditions were excluded: 1) cancer in the previous two years, 2) type-1 and type-2 diabetes, and 3) autoimmune diseases. Immediately after enrollment, trained staff collected anthropometric measurements and venous blood of study participants. The plasma fraction was separated from whole blood by centrifugation and stored at −80 °C until time of analysis. Smoking status was assessed as "ever smoked" or "never smoked". A previous complete description of this study can be found elsewhere [30]. Serum cytokines were quantified a previously described [32].

2.2. Plasma Extraction, Solid-Phase Extraction, and Isolation

The solvents used for extraction, isolation, and high performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS) analysis were either HPLC- or LC/MS-grade. Quantitative standard curves and deuterium labeled internal standard mixes and were made as previously described [35]. Extraction and isolation of non-esterified PUFA and oxylipids was performed as previously described [29,35], but modified as specified. In brief, plasma was thawed on ice. Approximately 500 μL plasma was transferred to a conical tube containing 1.5 mL HPLC-grade methanol and butylated hydroxytoluene (BHT) (100 μg/mL BHT). Next, 15 μL deuterium labeled internal standard mix and 7.4 μL LC/MS-grade formic acid were added to the plasma and methanol. Solid-phase extraction was performed for each sample using Phenomenex Strata-X (60 mg/3 mL, Phenomenex, Torrane, CA), as previously described [29]. The samples were capped under high-purity argon and stored at −20 °C for no longer than 7 days.

2.3. LC/MS/MS Analysis

Liquid chromatography separations were performed using an Ascentis Express C18 column (10 cm × 2.1 mm; 2.7 μm particles, Sigma-Aldrich, St. Louis, MO) maintained at 50 °C on a Waters ACQUITY UPLC system (Waters, Milford, MA). Mobile phase A was 0.1% formic acid in water and mobile phase B was acetonitrile. Analytes were eluted during a 15 min gradient using a flow rate of 0.3 mL/min. The mobile phase gradient began at 1% B, followed by a linear increase to 40% B at 2 min; then to 80% B at 8 min, and 99% B at 9 min, at which the composition was held until 13 min; then returned to 1% B until 15 min. The autosampler was cooled to 10 °C, and injection volume was set to 5 μL. The column was connected to a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA). Analytes were detected using electrospray ionization (ESI) with polarity switching between negative-ion mode (for oxylipids and PUFAs) and positive-ion mode (for endocannabinoids) using multiple reaction monitoring (MRM). Mass spectrometer parameters are outlined in Supplementary Table 1. TargetLynx (Waters) software was used for peak detection, integration and quantification.

2.4. Data Merging and Oxylipid Exclusion

The LC/MS/MS analyses quantified 62 PUFAs and oxylipids. The full list of analytes in our study, along with parent and daughter ion, oxylipid precursor PUFA, and oxylipid class is outlined in Supplementary Table 1. Since -omic data typically have a large proportion of missing values [36,37], analytes with >50% missing values were excluded from the analysis. The remaining 32 analytes in the data matrix consisted of: LA and LA-derived 9-hydroxyoctadecadienoic acid (HODE), 13-HODE, 9-oxooctadecadienoic acid (oxoODE), 13-oxoODE, 9,10-EpOME, 12,13-EpOME, 9,10-dihydroxyoctadecadienoic acid (DiHOME), and 12,13-DiHOME; ALA-derived 13-hydroxyoctadecatrienoic acid (HOTrE); DGLA-derived 15-hydroxyeicosatrienoic acid (HETrE); ARA and ARA-derived 5-HETE, 11-HETE, 12-HETE, 15-HETE, 20-HETE, 14,15-epoxyeicosatrienoic acid (EET), 8,9-dihydroxyeicosatrienoic acid (DHET), 11,12-DHET, 14,15-DHET, arachidonoyl ethanolamide (AEA), arachidonoyl glycerol (AG), leukotriene B4 (LTB4), prostaglandin E2 (PGE2), thromboxane B2 (TBX2); EPA and EPA-derived 5,6-dihydroxyeicosatetraenoic acid (DiHETE), 17,18-DiHETE; and DHA and DHA-derived 19,20-dihydroxydocosapentaenoic acid (HDPA) and docosahexaenoyl ethanolamide (DHEA).

2.5. Statistical Analyses

Our study was powered (0.8) to detect differences in plasma FAs, as previously described [33].The responses used in the statistical analyses were the anthropometrics: BMI, WC, and serum leptin, total adiponectin, IL-6, TNF-α, and C-peptide (continuous variables), and BMI category (categorical variable). The median, Q1, and Q3 concentrations were calculated for each plasma PUFA and oxylipid, which are outlined in Table 1. A significant proportion of LA, ARA, and 12,13-DiHOME concentrations were above (i.e., 10-fold) the highest standard curve, and there was not enough plasma for the re-extraction and reanalysis to quantify these three lipids. Therefore, the variables LA, ARA, and 12,13-DiHOME are reported as levels (i.e., peak area of compound/peak area of internal standard) in Table 1, so the relationship between these three lipids could be assessed with the responses. In this study, oxylipid totals and product to precursor ratios were calculated as follows: Total HODE = ∑9-HODE + 13-HODE; Total oxoODE = ∑oxoODE + 13-oxoODE; Total EpOME = ∑9,10-EpOME + 12,13-EpOME; Total HETE = ∑5-HETE + 11-HETE + 12-HETE + 15-HETE + 20-HETE; Total DHET = ∑8,9-DHET + 11,12-DHET + 14,15-DHET; Total DiHETE = ∑5,6-DiHETE + 17,18-DiHETE; 9-oxo/HODE = 9-oxoODE/9-HODE; 13-oxo/HODE = 13-oxoODE/13-HODE; 9,10-Di/EpOME = 9,10-DiHOME/9,10-EpOME; 12,13-Di/EpOME = 12,13-DiHOME/12,13-EpOME; 14,15-DH/EET = 14,15-DHET/14,15-EET.

Table 1 – Obesity is associated with increased plasma concentrations of monohydroxy arachidonic acid-derived oxylipids, and decreased plasma concentrations of eicosapentaenoic acid- and docosahexaenoic acid-derived vicinal diols (values expressed as median [Q1, Q3]).

Variable	Overall	Lean	Overweight	Obese	BH FDR p-value [c]
Sample size (n)	123	27	45	51	
Age (years)	58.0 [53.0, 60.5]	58.0 [55.5, 61.0][a]	54.0 [51.0, 59.0][b]	59.0 [54.0, 62.0][a]	$p \leq 0.005$
Ever smoked (% total)	31	25	24	27	
BMI (kg/m^2)	29.3 [26.1, 32.7]	23.6 [22.3, 24.4][a]	28.1 [27.1, 29.0][b]	33.7 [31.5, 36.7][c]	$p \leq 0.0001$
WC (in)	41.5 [37.0, 45.3]	33.5 [32.1, 36.8][a]	40.0 [37.3, 42.0][b]	47.0 [44.3, 49.5][c]	$p \leq 0.0001$
Leptin (ng/mL)	6.1 [3.3, 10.12]	2.1 [1.0, 3.5][a]	5.2 [3.0, 7.4][b]	13.4 [8.2, 25.1][c]	$p \leq 0.0001$
Adiponectin (μL/mL)	4.2 [3.2, 6.0]	5.4 [4.1, 7.7][a]	4.7 [3.7, 6.4][a]	3.9 [2.7, 5.0][b]	$p \leq 0.001$
IL-6 (pg/mL)	1.6 [0.0, 10.83]	0.3 [0.0, 11.4][a]	0.8 [0.0, 9.4][a]	3.5 [0.6, 9.5][a]	–
TNF-α (pg/mL)	7.3 [5.4, 9.8]	5.8 [4.2, 7.4][a]	7.3 [4.6, 8.8][a]	8.6 [6.5, 11.2][b]	$p \leq 0.001$
C-peptide (ng/mL)	2.5 [1.5, 4.0]	1.9 [1.1, 2.2][a]	2.1 [1.3, 3.4][a]	3.3 [2.5, 5.1][b]	$p \leq 0.0001$
Oxylipids (nmol/L)					
LA [a]	0.7 [0.4, 1.0]	0.5 [0.4, 1.0][a]	0.7 [0.4, 1.2][a]	0.7 [0.5, 1.0][a]	–
9-HODE	14.3 [9.5, 23.9]	17.1 [10.0, 30.9][a]	14.4 [9.8, 21.5][a]	13.5 [9.3, 25.1][a]	–
13-HODE	13.4 [8.6, 22.0]	17.4 [8.3, 25.7][a]	13.4 [9.0, 19.1][a]	10.8 [8.3, 22.6][a]	–
Total HODE	29.3 [19.1, 47.4]	38.6 [19.2, 52.4][a]	29.3 [19.2, 39.6][a]	24.2 [17.6, 51.9][a]	–
9-oxoODE	7.9 [5.1, 12.0]	7.7 [5.1, 11.4][a]	8.5 [5.8, 11.7][a]	8.3 [5.0, 12.2][a]	–
13-oxoODE	2.2 [1.4, 3.8]	2.2 [1.5, 3.3][a]	2.2 [1.3, 4.1][a]	2.3 [1.5, 3.8][a]	–
Total oxoODE	11.0 [6.7, 15.7]	11.1 [7.3, 15.3][a]	11.0 [6.7, 16.4][a]	11.0 [6.7, 15.2][a]	–
9-oxo/HODE	0.5 [0.4, 0.7]	0.5 [0.3, 0.6][a]	0.6 [0.5, 0.8][a]	0.5 [0.3, 0.6][a]	–
13-oxo/HODE	0.3 [0.1, 0.2]	0.1 [0.1, 0.2][a]	0.2 [0.1, 0.2][a,b]	0.2 [0.1, 0.2][b]	0.083
9,10-EpOME	21.6 [15.0, 32.7]	19.8 [15.3, 32.1][a]	19.8 [14.4, 34.2][a]	24.0 [15.9, 30.0][a]	–
12,13-EpOME	40.2 [27.0, 57.9]	40.2 [28.5, 76.5][a]	47.4 [25.2, 60.0][a]	37.8 [26.1, 52.8][a]	–
Total EpOME	64.2 [42.6, 94.2]	70.8 [41.7, 102.0][a]	65.4 [42.6, 100.2][a]	60.6 [42.6, 84.0][a]	–
9,10-DiHOME	8.4 [4.7, 16.4]	9.0 [5.7, 39.6][a]	10.8 [4.8, 17.4][a]	6.6 [4.1, 13.1][a]	–
12,13-DiHOME [a]	3.3 [2.1, 5.7]	4.2 [1, 10.8][a]	4.8 [2.63, 6.17][a]	2.7 [1.6, 3.9][b]	$p \leq 0.05$
Total DiHOME	12.0 [7.1, 23.2]	13.9 [8.7, 53.0][a]	16.7 [7.8, 24.9][a]	9.8 [6.2, 18.6][b]	0.061
9,10-Di/EpOME	0.4 [0.2, 0.9]	0.5 [0.3, 1.3][a]	0.5 [0.3, 1.0][a,b]	0.3 [0.2, 0.7][b]	0.065
12,13-Di/EpOME	0.1 [0.1, 0.1]	0.1 [0.1, 0.1][a]	0.1 [0.1, 0.2][a]	0.1 [0.0, 0.1][b]	$p \leq 0.05$
13-HOTrE	2.0 [1.1, 3.9]	3.2 [1.5, 4.4][a]	1.8 [1.2, 3.0][b]	1.8 [1.1, 3.5][a]	–

15-HETrE	0.4 [0.2, 0.6]	0.2 [0.2, 0.5][a]	0.3 [0.2, 0.5][a]	0.5 [0.3, 0.7][b]	$p \leq 0.05$
ARA [a]	14.9 [10.4, 21.4]	13.5 [9.6, 17.3][a]	14.6 [10.1, 22.9][a]	16.0 [12.7, 22.3][a]	–
5-HETE	2.8 [1.6, 9.5]	1.5 [0.8, 6.2][a]	2.4 [1.7, 5.0][a,b]	4.5 [2.1, 12.0][b]	$p \leq 0.05$
11-HETE	0.7 [0.3, 1.3]	0.4 [0.2, 0.8][a]	0.5 [0.3, 0.9][a]	0.9 [0.5, 1.7][b]	$p \leq 0.05$
12-HETE	9.4 [6.0, 15.3]	7.5 [3.8, 14.1][a]	8.5 [6.2, 13.6][a,b]	11.9 [6.7, 17.8][b]	–
15-HETE	0.8 [0.5, 1.2]	0.8 [0.4, 1.0][a]	0.6 [0.5, 1.1][a,b]	1.0 [0.7, 1.5][b]	$p \leq 0.05$
20-HETE	0.8 [0.5, 1.2]	0.5 [0.3, 0.9][a]	0.7 [0.4, 1.0][a,b]	0.9 [0.6, 1.3][b]	$p \leq 0.05$
Total HETE	19.0 [10.9, 32.8]	19.4 [8.9, 25.8][a]	16.5 [11.2, 22.7][a]	22.4 [11.3, 39.5][a]	–
14,15-EET	1.3 [1.0, 2.2]	1.2 [0.5, 1.8][a]	1.3 [1.0, 2.3][a]	1.9 [1.1, 2.2][a]	–
8,9-DHET	0.6 [0.4, 0.8]	0.6 [0.3, 0.8][a]	0.6 [0.5, 0.8][a]	0.5 [0.4, 0.9][a]	–
11,12-DHET	1.0 [0.8, 1.4]	1.0 [0.7, 1.5][a]	1.0 [0.8, 1.4][a]	1.1 [0.8, 1.4][a]	–
14,15-DHET	1.6 [1.3, 2.0]	1.5 [1.3, 2.3][a]	1.6 [1.2, 2.1][a]	1.6 [1.3, 1.9][a]	–
Total DHET	3.2 [2.5, 4.2]	3.0 [2.4, 4.8][a]	3.2 [2.6, 4.2][a]	3.2 [2.6, 4.0][a]	–
14,15-DH/EET	1.3 [0.9, 1.8]	1.5 [1.3, 2.7][a]	1.3 [1.0, 1.9][a]	1.1 [0.9, 1.5][a]	–
AEA	0.2 [0.2, 0.2]	0.2 [0.1, 0.2][a]	0.2 [0.1, 0.2][a]	0.2 [0.2, 0.2][a]	–
AG	1.4 [0.8, 2.0]	1.3 [0.8, 1.6][a]	1.6 [0.8, 1.9][a]	1.4 [0.9, 2.1][a]	–
LTB4	0.3 [0.2, 0.5]	0.2 [0.2, 0.4][a]	0.3 [0.2, 0.6][a]	0.3 [0.2, 0.5][a]	–
PGD2	4.4 [2.2, 10.3]	6.6 [3.3, 12.1][a]	4.1 [2.4, 13.4][a]	4.2 [2.0, 6.6][a]	–
TBX2	0.3 [0.2, 0.9]	0.3 [0.2, 0.6][a]	0.5 [0.2, 1.3][a]	0.3 [0.2, 0.8][a]	–
EPA [b]	0.2 [0.1, .3]	0.2 [0.1, 0.5][a]	0.2 [0.1, .4][a]	0.2 [0.1, 0.3][a]	–
5,6-DiHETE	5.0 [3.0, 7.3][a]	5.7 [3.5, 12.2][a]	5.3 [2.5, 7.2][a]	4.6 [3.2, 6.0][a]	–
17,18-DiHETE	8.5 [5.9, 14.3]	14.8 [9.8, 23.5][a]	8.0 [5.9, 14.6][a]	6.8 [5.0, 9.2][b]	$p \leq 0.0005$
Total DiHETE	13.4 [9.5, 25.0]	23.1 [12.7, 37.5][a]	13.2 [9.0, 24.7][a]	10.9 [9.3, 18.6][a]	$p \leq 0.05$
DHA [b]	4.6 [3.3, 7.8]	5.6 [3.4, 8.3][a]	4.6 [3.2, 8.1][a]	4.5 [3.5, 7.3][a]	–
19,20-HDPA	2.8 [2.1, 4.4]	4.5 [3.1, 5.7][a]	3.3 [2.2, 5.0][b]	2.5 [1.6, 3.0][c]	$p \leq 0.0005$
DHEA	0.5 [0.3,0.8]	0.6 [0.5,1.1][a]	0.5 [0.5,0.7][a]	0.5 [0.2, 0.8][a]	–

Adiponectin concentrations were calculated as follows ∑low + middle + high molecular weight adiponectin isoforms. Oxylipid totals were calculated as follows: Total HODE calculated as ∑9-HODE + 13-HODE; Total oxoODE calculated as ∑9-oxoODE + 13-oxoODE; Total EpOME calculated as ∑9,10-EpOME + 12,13-EpOME; Total DiHOME calculated as ∑9,10-DiHOME 12,13-DiHOME; Total HETE calculated as ∑5-HETE + 11-HETE + 12-HETE + 15-HETE + 20-HETE; Total DHET calculated as ∑8,9-DHET + 11,12-DHET + 14,15-DHET; Total DiHETE calculated as ∑5,6-DiHETE + 17,18-DiHETE. Oxylipid product-to-precursor ratios are calculated as follows: 9-oxo/HODE = 9-oxoODE/9-HODE; 13-oxo/HODE = 13-oxoODE/13-HODE; 9,10-Di/EpOME = 9,10-DiHOME/9,10-EpOME; 12,13-Di/EpOME = 12,13-DiHOME/12,13-EpOME; 14,15-DH/EET = 14,15-DHET/14,15-EET.

[a] Plasma non-esterified LA, 12,13-DiHOME, and ARA values are presented as levels (i.e., peak area of compound/peak area of internal standard), since a majority of participant's concentrations were greater (i.e., >10 fold) than the highest standard curve value.

[b] Plasma non-esterified EPA and DHA are expressed as μmol/L.

[c] Categorical analysis of BMI by Kruskal–Wallis one-way ANOVA and Dunn's test for multiple comparison. *p*-Values were corrected for false discovery rate (FDR) according to Benjamini–Hochberg (BH).

Statistical differences in non-esterified PUFA and oxylipid concentrations, levels, and product-to-precursor ratios (Table 1), between BMI categories were determined using Kruskal–Wallis non-parametric one-way ANOVA with Dunn's test for multiple comparison. Since a majority (>70%) of PUFAs and oxylipids were not normally distributed, the data matrix was log transformed for parametric analyses. For regressions, responses were regressed on oxylipids as well as clinical covariates including age of the participant and the smoking status "ever smoked" or "never smoked". The linear relationship between responses and plasma lipids was assessed by regressing responses on log transformed PUFAs, oxylipids, and oxylipid parent-to-precursor ratios individually (i.e., one at a time). Next, to determine whether differences in oxylipid concentrations were due to metabolism or altered PUFA availability (i.e., substrate levels), each participants oxylipid concentration was normalized to their concentration or level of each PUFA, respectively. Responses were then regressed on log transformed PUFA-normalized oxylipids individually (i.e, one at a time). Finally, for oxylipids significant across multiple statistical tests, the concentrations of these oxylipids were categorized into tertiles (referenced against the lowest category) for obese relative to lean, using polytomous logistic regressions as previously described [38]. The test for trend was carried out for the oxylipid of interest across tertiles, respectively. In all

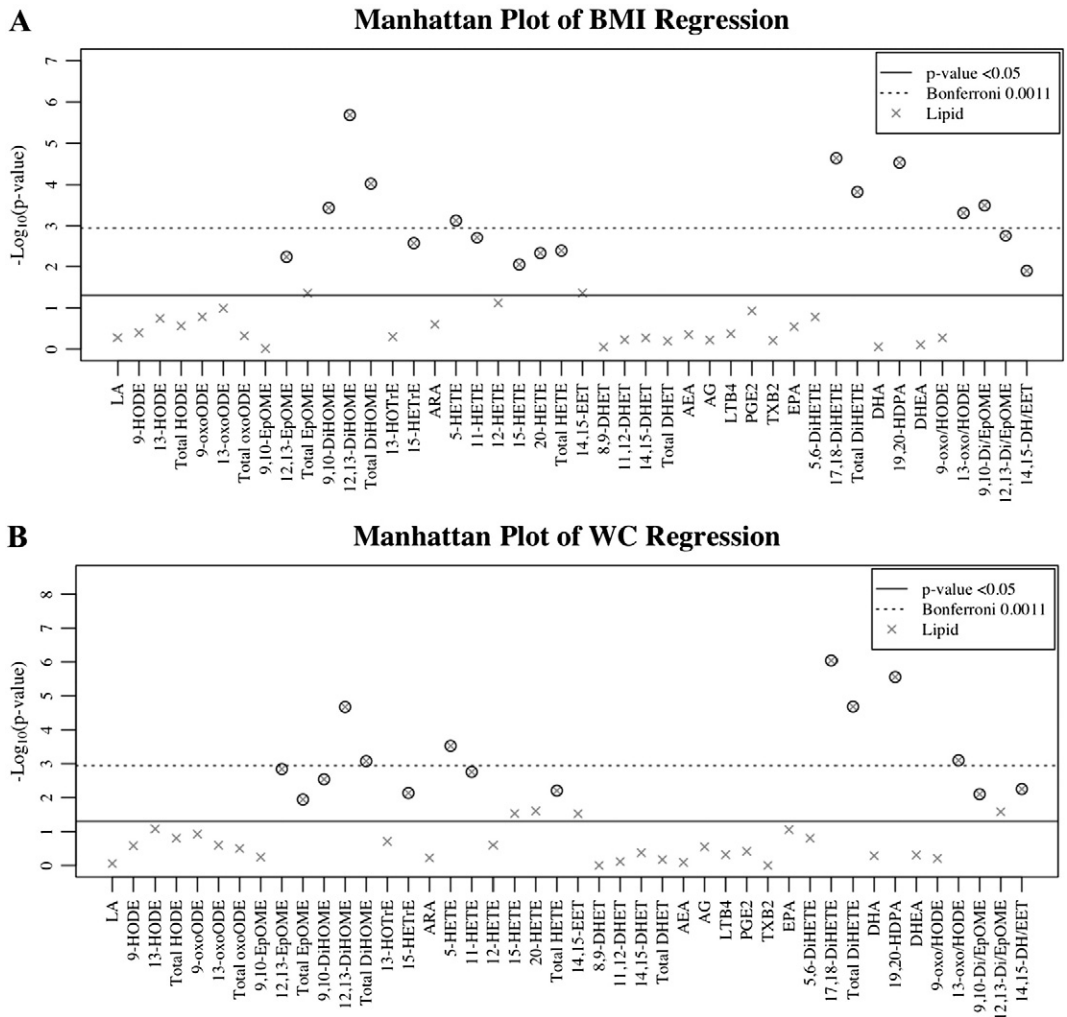

Fig. 1 – Manhattan plots of the –log10(*p*-value) for the responses body mass index (BMI), waist circumference (WC), and serum leptin, C-peptide, and total adiponectin regressed on log transformed PUFAs, oxylipids, and oxylipid parent-to-precursor ratios individually (i.e., one at a time). Single non-esterified plasma fatty acid, oxylipid, and product to precursor ratio regression models are defined as: $\text{Response}_i = \text{age}_i + \text{smoking}_i + \log(\text{lipid}_{ij})$, where $i = (1,\ldots,123)$ and $j = (1,\ldots,44)$. Each *y*-axis represents –log10(*p*-value) for each respective model, and *x*-axis represents the plasma non-esterified fatty acid, oxylipid, and oxylipid product-to-precursor ratios. Plasma lipids with Benjamini–Hochberg false discovery rate *p*-values ≤0.05 are circled. Oxylipids totals calculated as follows: Total HODE = ∑9-HODE + 13-HODE; Total oxoODE = ∑9-oxoODE + 13-oxoODE; Total EpOME = ∑9,10-EpOME + 12,13-EpOME; Total HETE = ∑5-HETE + 11-HETE + 12-HETE + 15-HETE + 20-HETE; Total DHET = ∑8,9-DHET + 11,12-DHET + 14,15-DHET; Total DiHETE = ∑5,6-DiHETE + 17,18-DiHETE; 9-oxo/HODE = 9-oxoODE/9-HODE; 13-oxo/HODE = 13-oxoODE/13-HODE; 9,10-Di/EpOME = 9,10-DiHOME/9,10-EpOME; 12,13-Di/EpOME = 12,13-DiHOME/12,13-EpOME; 14,15-DH/EET = 14,15-DHET/14,15-EET.

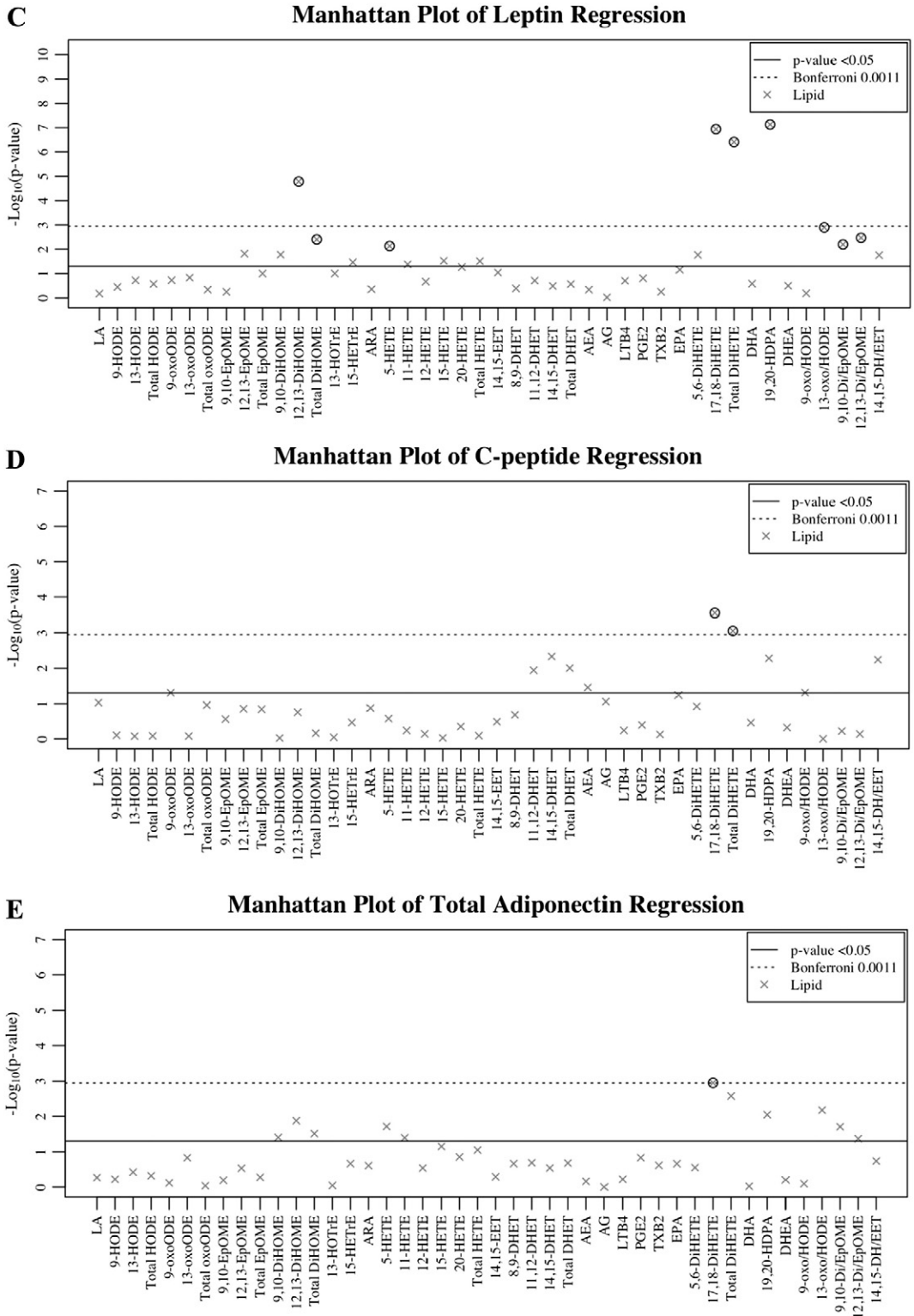

Fig. 1 – *(continued.)*

statistical analyses, missing PUFA and oxylipid values were imputed as the $\sqrt{minimum\ value}$ for each respective PUFA and oxylipid. *p*-Values were corrected for false discovery rate (FDR) according to Benjamini–Hochberg (BH) and Bonferroni [39,40]. Statistical analyses of data were performed using R v3.2.2 [41], except polytomous logistic regressions which were performed using SAS version 9.4 (SAS, Cary, NC), as we have previously described [30–33]. All statistical codes used in analyses are publicly available at https://github.com/AustinPickens/Targeted-Lipidomics.

Table 2 – Estimated effects of plasma non-esterified fatty acids and oxylipids from single lipid regression models.

	BMI		WC		Leptin		C-peptide		Total Adiponectin	
Oxylipid	Beta	p-Value	Beta	p-Value	Beta	p-Value	Beta	p-Value	Beta	p-Value
LA	0.52	–	0.14	–	0.07	–	−0.16	–	−0.21	–
9-HODE	−0.59	–	−0.89	–	−0.13	–	−0.02	–	0.16	–
13-HODE	−0.91	–	−1.35	–	−0.18	–	−0.02	–	0.26	–
Total HODE	−0.76	–	−1.12	–	−0.15	–	−0.02	–	0.21	–
9-oxoODE	−0.97	–	−1.24	–	−0.18	–	−0.16	$p \leq 0.05$	0.09	–
13-oxoODE	1.08	–	0.87	–	0.19	–	−0.02	–	−0.41	–
Total oxoODE	0.22	–	0.26	–	−0.11	–	−0.14	–	−0.04	–
9,10-EpOME	0.04	–	−0.58	–	0.1	–	−0.11	–	−0.18	–
12,13-EpOME	−2.11	$p \leq 0.01$	−2.78	$p \leq 0.005$	−0.37	$p \leq 0.05$	−0.13	–	0.35	–
Total EpOME	−1.69	$p \leq 0.05$	−2.42	$p \leq 0.05$	−0.28	–	−0.14	–	0.23	–
9,10-DiHOME	−1.70	$p \leq 0.001$	−1.64	$p \leq 0.005$	−0.23	$p \leq 0.05$	0.00	–	0.42	$p \leq 0.05$
12,13-DiHOME	−2.53	$p \leq 0.0001$	−2.62	$p \leq 0.0001$	−0.46	$p \leq 0.0001$	−0.09	–	0.58	$p \leq 0.05$
Total DiHOME	−1.95	$p \leq 0.0001$	−1.93	$p \leq 0.0001$	−0.29	$p \leq 0.005$	−0.02	–	0.47	$p \leq 0.05$
13-HOTrE	−0.35	–	−0.77	–	−0.17	–	0.01	–	0.03	–
15-HETrE	1.94	$p \leq 0.005$	1.99	$p \leq 0.01$	0.28	$p \leq 0.05$	0.07	–	−0.34	–
ARA	0.89	–	0.47	–	0.12	–	−0.13	–	−0.38	–
5-HETE	1.35	$p \leq 0.005$	1.65	$p \leq 0.005$	0.22	$p \leq 0.01$	0.05	–	−0.40	$p \leq 0.05$
11-HETE	1.54	$p \leq 0.005$	1.78	$p \leq 0.005$	0.21	$p \leq 0.05$	0.03	–	−0.44	$p \leq 0.05$
12-HETE	1.00	*0.076*	0.75	–	0.14	–	−0.02	–	−0.26	–
15-HETE	1.60	$p \leq 0.01$	1.53	$p \leq 0.05$	0.27	$p \leq 0.05$	0.01	–	−0.47	*0.071*
20-HETE	2.28	$p \leq 0.005$	2.09	$p \leq 0.05$	0.31	*0.054*	−0.07	–	−0.51	–
Total HETE	0.54	–	0.66	–	0.26	$p \leq 0.05$	0.02	–	−0.43	*0.089*
14,15-EET	1.47	$p \leq 0.05$	1.82	$p \leq 0.05$	0.25	–	0.08	–	−0.21	–
8,9-DHET	−0.15	–	0.00	–	−0.17	–	−0.15	–	0.55	–
11,12-DHET	−0.60	–	−0.37	–	−0.29	–	−0.32	$p \leq 0.05$	0.59	–
14,15-DHET	−0.79	–	−1.16	–	−0.25	–	−0.40	$p \leq 0.005$	0.57	–
Total DHET	−0.90	–	−1.47	–	−0.28	–	−0.36	$p \leq 0.05$	0.67	–
AEA	0.97	–	0.35	–	0.19	–	−0.30	$p \leq 0.05$	−0.22	–
AG	−0.39	–	−0.91	–	0.01	–	0.14	*0.088*	0.01	–
LTB4	0.52	–	0.53	–	0.17	–	−0.04	–	−0.15	–
PGD2	−0.69	–	−0.44	–	−0.13	–	−0.04	–	0.27	–
TBX2	0.28	–	0.00	–	−0.06	–	−0.02	–	0.27	–
EPA	−0.46	–	−0.83	*0.087*	−0.15	*0.070*	−0.09	*0.0581*	0.22	–
5,6-DiHETE	−0.70	–	−0.82	–	−0.24	$p \leq 0.05$	−0.09	–	0.23	–
17,18-DiHETE	−2.43	$p \leq 0.0001$	−3.19	$p \leq 0.0001$	−0.6	$p \leq 0.0001$	−0.24	$p \leq 0.0005$	0.81	$p \leq 0.005$
Total DiHETE	−1.39	$p \leq 0.005$	−1.69	$p \leq 0.001$	−0.67	$p \leq 0.0001$	−0.26	$p \leq 0.001$	0.87	$p \leq 0.005$
DHA	0.11	–	−0.54	–	−0.16	–	−0.08	–	0.02	–
19,20-HDPA	−3.52	$p \leq 0.0001$	−4.48	$p \leq 0.0001$	−0.89	$p \leq 0.0001$	−0.27	$p \leq 0.01$	0.96	$p \leq 0.01$
DHEA	−0.23	–	−0.64	–	−0.16	–	−0.07	–	0.17	–
9-Oxo/HODE	−0.51	–	−0.46	–	−0.07	–	−0.18	$p \leq 0.05$	−0.09	–
13-Oxo/HODE	2.63	$p \leq 0.0005$	2.91	$p \leq 0.001$	0.49	$p \leq 0.0005$	0.00	–	−0.88	$p \leq 0.01$
9,10-Di/EpOME	−1.72	$p \leq 0.0005$	−1.47	$p \leq 0.01$	−0.26	$p \leq 0.01$	0.03	–	0.48	$p \leq 0.05$
12,13-Di/EpOME	−2.03	$p \leq 0.005$	−1.67	$p \leq 0.05$	−0.38	$p \leq 0.005$	−0.03	–	0.57	$p \leq 0.05$
14,15-DH/EET	−1.89	$p \leq 0.05$	−2.40	$p \leq 0.01$	−0.36	$p \leq 0.05$	−0.24	$p \leq 0.01$	0.43	–

Beta coefficients and *p*-values were determined by regressing responses on log transformed PUFAs, oxylipids, and oxylipid parent-to-precursor ratios individually (i.e., one at a time). Responses were defined as body mass index (BMI), waist circumference (WC), and serum log transformed leptin, log transformed C-peptide, and total adiponectin. Single non-esterified plasma fatty acid, oxylipid, and product to precursor ratio regression models are defined as: $\text{Response}_i = \text{age}_i + \text{smoking}_i + \log(\text{lipid}_{ij})$, where $i = (1,\ldots,123)$ and $j = (1,\ldots,44)$. *p*-Values are italicized if $0.05 > p < 0.09$. Oxylipids totals calculated as follows: Total HODE = ∑9-HODE + 13-HODE; Total oxoODE = ∑9-oxoODE + 13-oxoODE; Total EpOME = ∑9,10-EpOME + 12,13-EpOME; Total HETE = ∑5-HETE + 11-HETE + 12-HETE + 15-HETE + 20-HETE; Total DHET = ∑8,9-DHET + 11,12-DHET + 14,15-DHET; Total DiHETE = ∑5,6-DiHETE + 17,18-DiHETE; 9-oxo/HODE = 9-oxoODE/9-HODE; 13-oxo/HODE = 13-oxoODE/13-HODE; 9,10-Di/EpOME = 9,10-DiHOME/9,10-EpOME; 12,13-Di/EpOME = 12,13-DiHOME/12,13-EpOME; 14,15-DH/EET = 14,15-DHET/14,15-EET.

3. Results

3.1. *Characteristics and Oxylipid Concentrations of Study Participants*

Median, Q1, and Q3 values of age, smoking, anthropometric, serum cytokines, and non-esterified PUFAs and oxylipid concentrations for the overall population ($n = 123$) and separated by BMI category are presented in Table 1. Non-parametric ANOVAs were conducted for each variable in Table 1, across BMI categories, and BH FDR corrected *p*-values are presented. In brief, lean and obese participants were older that overweight participants. As expected, BMI, WC, and serum leptin, IL-6, and TNF-α increased with increasing BMI category. Serum total adiponectin was lower in obese

participants compared to overweight and lean participants. Concentrations of LA-derived oxylipids did not differ across BMI category, however, 12,13-DiHOME and 12,13-Di/EpOME levels were decreased in obese individuals compared to both overweight and lean (Table 1). DGLA-derived 15-HETrE concentrations were significantly elevated in obese individuals compared to both overweight and lean (Table 1). ARA-derived 5-, 15-, and 20-HETE concentrations were higher in obese individuals compared to lean, and 11-HETE concentrations were elevated in obesity compared to both overweight and lean. 12-HETE concentrations were significantly higher in obese individuals compared to lean in the multiple comparison test, but the model p-value was not significant after p-value correction (BH FDR $p = 0.1141$, data not shown). EPA-derived 17,18-DiHETE concentrations were decreased in obese individuals compared to both overweight and lean. DHA-derived 19,20-HDPA was significantly decreased in obese individuals compared to both overweight and lean, and also decreased in overweight individuals compared to lean (Table 1).

3.2. Obesity is Associated with Increased Plasma HETEs and Decreased Vicinal Diols

Next, responses (i.e., anthropometrics and serum cytokines) were regressed on PUFAs, oxylipids, and oxylipid product-to-precursor ratios individually, to determine the estimated effects of each lipid for each response (Fig. 1 and Table 2). Models were adjusted for age and smoking. p-Values from single lipid regressions that were significant after BH FDR correction are circled, and p-values significant below Bonferroni cutoff ($p < 0.0011$) are displayed above the hashed line in Manhattan plots (Fig. 1). In general, the oxylipids significantly associated with obesity after FDR correction consisted of LA-derived epoxides, ARA-derived HETEs, and LA-, EPA-, and DHA-derived vicinal diols. In fact, the majority of oxylipids significant below the Bonferroni cutoff, across models, were vicinal diols (Fig. 1). In general, BMI, WC, and serum leptin were either inversely or positively associated with similar non-esterified oxylipids (Table 2). BMI, WC, and leptin were inversely associated with LA-derived 12,13-EpOME, 9,10-DiHOME, 12,13-DiHOME, and Total DiHOME. DGLA-derived 15-HETrE and ARA-derived oxylipids 5-, 11-, 15-, and 20-HETE had positive relationships with BMI, WC, and leptin. EPA- and DHA-derived vicinal diols were inversely associated with BMI, WC, leptin, and C-peptide. To assess whether oxylipid differences were a result of altered metabolism, responses were regressed on oxylipid product-to-precursor ratios (Fig. 1 and Table 2). Neither LA-derived 13-oxoODE nor 13-HODE were significantly associated with responses when analyzed individually, however, increased ratios of 13-oxoODE to 13-HODE (13-oxo/HODE) were associated with increased BMI, WC, and leptin concentrations (Table 2). The ratio of vicinal diols normalized to their precursor epoxide had inverse associations with BMI and WC. Increased ratios of LA-derived 9,10-Di/EpOME, 12,13-Di/–EpOME, and ARA-derived 14,15-DH/EET were associated with decreased BMI, WC, and leptin. Adiponectin is an anti-inflammatory cytokine inversely associated with obesity [42], therefore, it was expected that adiponectin would be:

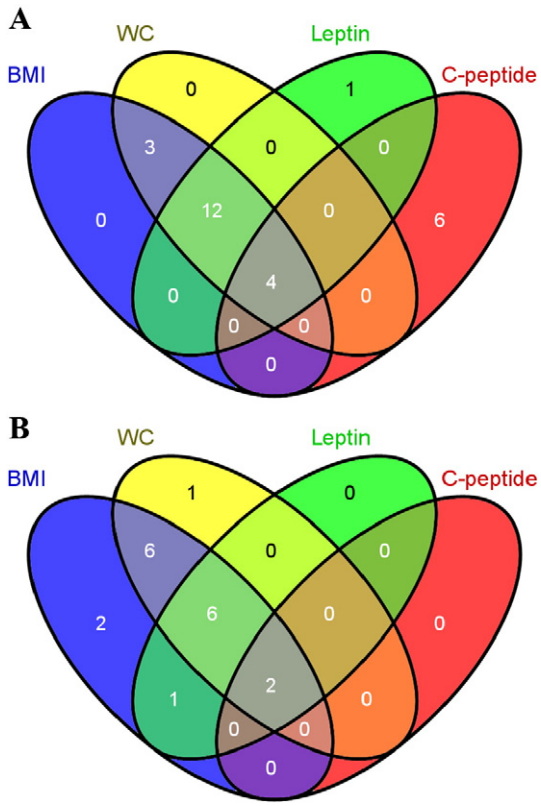

Fig. 2 – Venn diagrams displaying the relationship of non-esterified lipids significantly associated between the responses body mass index (BMI), waist circumference (WC), leptin, and C-peptide. (A) Relationship of lipids with p-values <0.05 for the responses. (B) Relationship of lipids with Benjamini–Hochberg p-values <0.05.

positively associated with LA-, EPA-, and DHA-derived vicinal diols; inversely associated with 5-, 11-, 15-HETE; and positively associated with the ratio of vicinal diols to precursor epoxides. The proinflammatory serum cytokines IL-6 and TNF-α were not significantly associated with non-esterified lipids (data not shown).

3.3. Vicinal Diols and HETEs are Associated Across Responses

Next, lipids significant across several traits from single lipid regression were inputted into the software Venny [43], to illustrate the lipids significant between several responses using Venn diagrams. Since Venny creates a Venn diagram for up to 4 traits, the anthropometrics BMI and WC, and the serum cytokines leptin and C-peptide were selected for the illustration. Relationships between the traits and lipids with p-values <0.05 are presented in Fig. 2A. There were several lipids with p-values <0.05 associated with traits (Figs. 1 and 2A), for instance: 19 lipids with BMI, 19 lipids with WC, 17 lipids with leptin, and 10 lipids with C-peptide. In total, there were 4 lipids associated between the four traits (Fig. 2A) which included 17,18-DiHETE, Total DiHETE, 19,20-HDPA, and 14,15-DH/EET. A Venn diagram of only lipids with BH FDR p-values <0.05 for the traits are presented in Fig. 2B. There were 2 lipids with BH FDR p-values <0.05 associated between the four traits (Fig. 2B), and those lipids were: 17,18-DiHETE and Total DiHETE.

3.4. PUFA Normalized 5- and 11-HETE, and Vicinal Diols are Associated with Obesity

Given that PUFAs are obtained through dietary intake, and PUFAs serve as substrates for oxylipids, each oxylipid was then normalized to its parent PUFA. This was performed to assess whether our associations between anthropometrics and serum cytokines, and plasma oxylipids were, in fact, due to altered levels of PUFA substrates or altered metabolic pathways affecting oxylipid production. Each patient's LA- and ARA-derived oxylipids were normalized to their LA and ARA levels, respectively, and each patient's EPA- and DHA-derived oxylipids were normalized to their EPA and DHA μmol/L concentrations, respectively. Responses were regressed on PUFA-normalized oxylipids, and models were adjusted for age and smoking. *p*-Values from single regressions that were significant after BH FDR correction are circled, and *p*-values significant below Bonferroni cutoff ($p < 0.0015$) are displayed above the hashed line in Manhattan plots (Fig. 3). The regression results for BMI and WC were similar. After normalization to LA, the oxylipids 12,13-EpOME, Total EpOME, 9,10-DiHOME, 12,13-DiHOME, and Total DiHOMEs were inversely associated with BMI and WC. ARA-derived oxylipids were positively associated with BMI and WC after normalization with ARA, in particular, 5-HETE and 11-HETE (Fig. 3). BMI and WC were both inversely associated with DHA normalized 19,20-HDPA concentrations. After FDR *p*-value correction, leptin was only associated with the vicinal diols 12,13-DiHOME (LA-derived) and 19,20-HDPA (DHA-derived). C-peptide and total adiponectin were not significantly associated with PUFA-normalized non-esterified lipids after FDR *p*-value correction (Fig. 3). The proinflammatory serum cytokines IL-6 and TNF-α were not significantly associated with PUFA-normalized non-esterified lipids (data not shown).

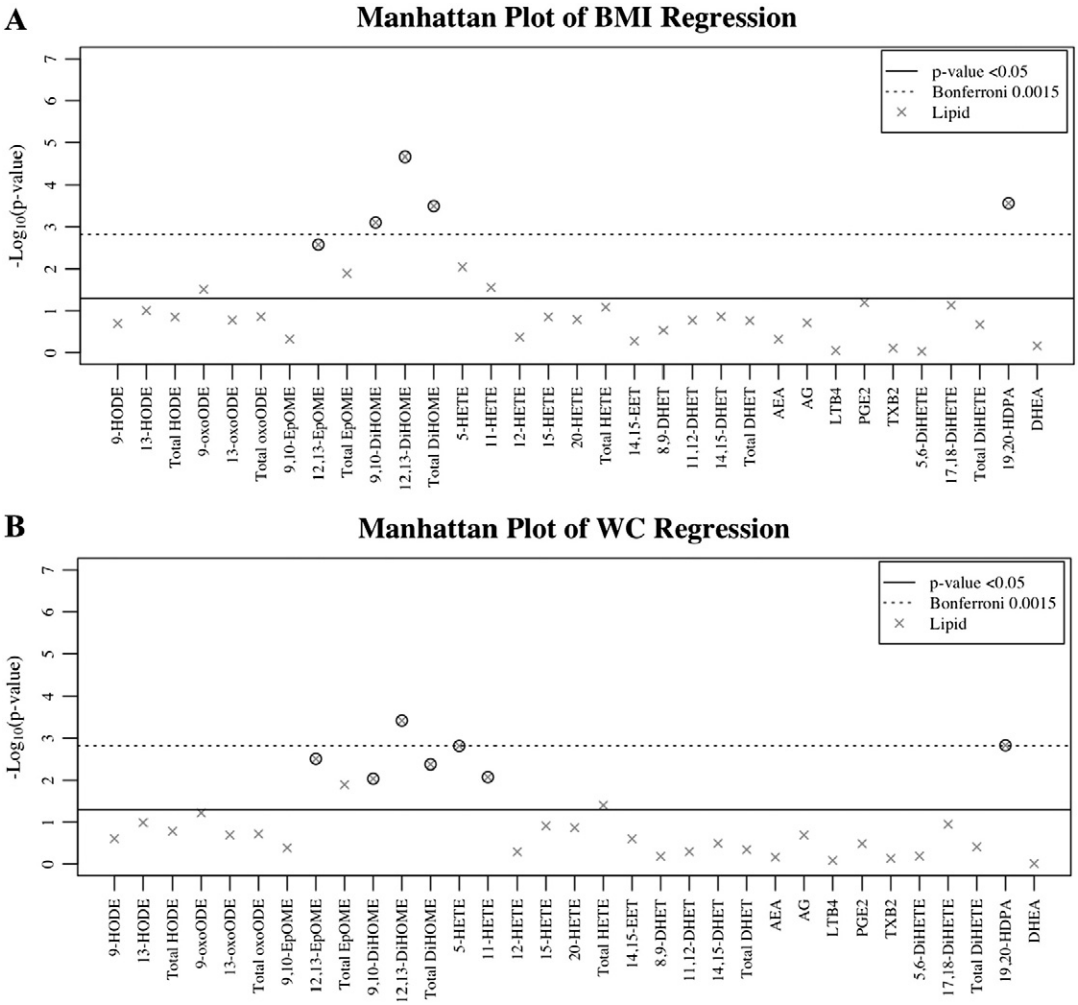

Fig. 3 – Manhattan plots of the −log10(*p*-value) for the responses body mass index (BMI), waist circumference (WC), and serum leptin, C-peptide, and total adiponectin regressed on log transformed PUFA-normalized oxylipids (i.e., 9,10-DiHOME/LA and 5-HETE/ARA) and oxylipid totals individually (i.e., one at a time). Single non-esterified plasma PUFA-normalized oxylipid models are defined as: $\text{Response}_i = \text{age}_i + \text{smoking}_i + \log(\text{lipid}_{ic})$, where i = (1,…,123) and c = (1,…,33). Each *y*-axis represents −log10(*p*-value) for each respective model, and *x*-axis represents the plasma non-esterified oxylipid or fatty acid. Plasma lipids with Benjamini–Hochberg false discovery rate *p*-values ≤0.05 are circled. Oxylipids totals calculated as follows: Total HODE = (∑9-HODE + 13-HODE)/LA; Total oxoODE = (∑9-oxoODE + 13-oxoODE)/LA; Total EpOME = (∑9,10-EpOME + 12,13-EpOME)/LA; Total HETE = (∑5-HETE + 11-HETE + 12-HETE + 15-HETE + 20-HETE)/ARA; Total DHET = (∑8,9-DHET + 11,12-DHET + 14,15-DHET)/ARA; Total DiHETE = (∑5,6-DiHETE + 17,18-DiHETE)/EPA.

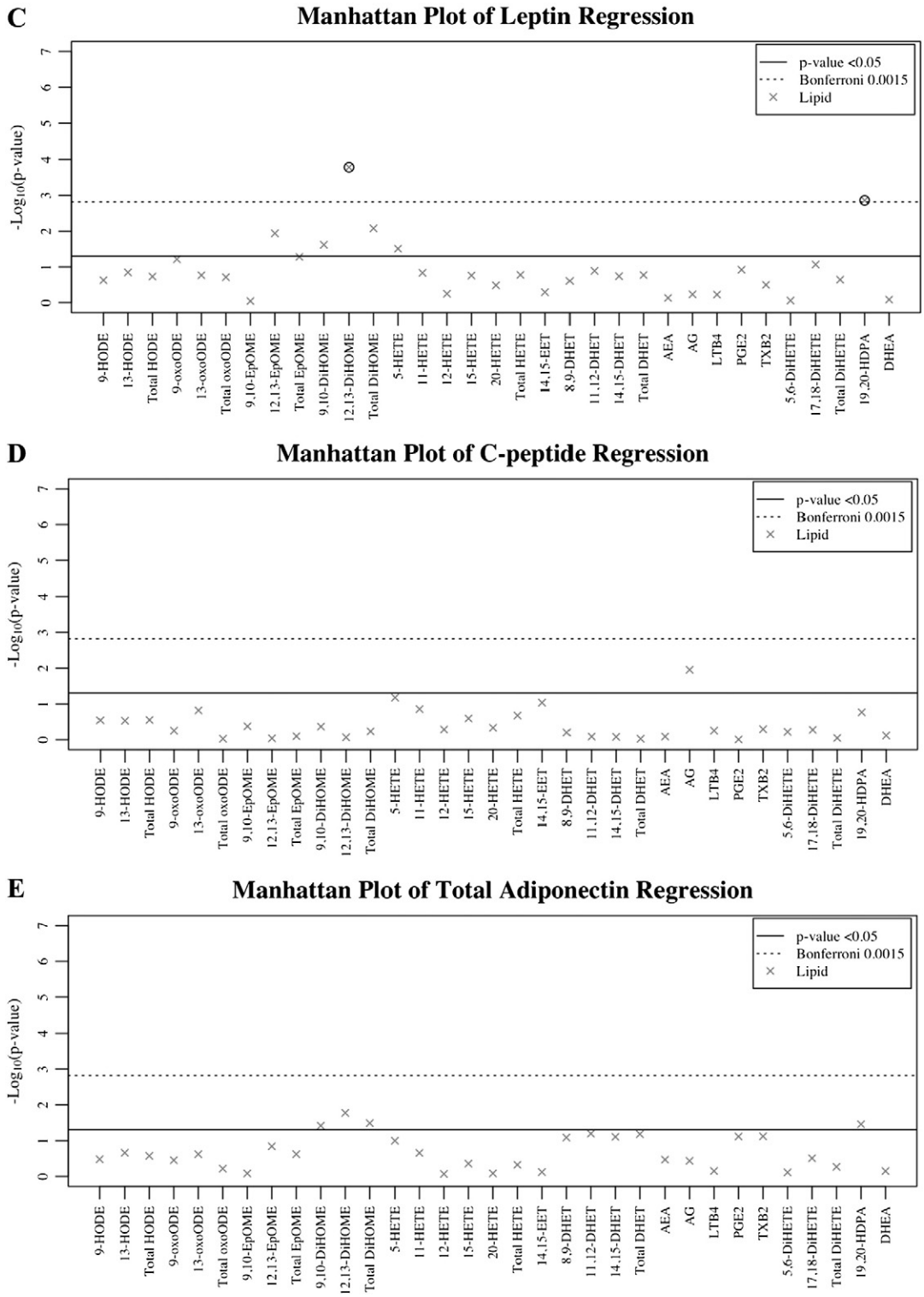

Fig. 3 – *(continued.)*

3.5. *Obesity is Associated with Specific Concentrations of Oxylipids*

Finally, since oxylipids concentration ranges are not well characterized in obesity, we separated several of our highly significant epoxides, HETEs, and vicinal diols into tertiles, to determine concentration ranges that are most likely to be associated with obesity. The response in polytomous logistic regressions was BMI category and models were adjusted for age and smoking. Categorical increases for 9,10-EpOME, 12,13-EpOME, and 9,10-DiHOME were not significantly associated with obesity compared to lean individuals (Table 3). However, for each category increase in 5-HETE, an individual was 2.57 times more likely to be obese rather than lean. Participants

Table 3 – Associations of non-esterified plasma oxylipids, as tertiles, with being obese relative to lean.

Plasma concentration [a]	Test for exposure OR [95% CI] [b]	Test for trend OR (p trend) [c]
9,10-EpOME (nmol/L)		1.21
≤16.2	1	(0.5185)
>16.2 to ≤28.2	1.45 [0.46, 4.54]	
>28.2	1.46 [0.45, 4.79]	
12,13-EpOME (nmol/L)		0.79
≤29.6	1	(0.4211)
>29.6 to ≤51.9	1.11 [0.35, 3.51]	–
>51.9	0.61 [0.19, 1.93]	–
9,10-DiHOME (nmol/L)		0.64
≤5.7	1	(0.1463)
>5.7 to ≤12.9	0.72 [0.26, 3.16]	
>12.9	0.41 [0.13, 1.36]	
5-HETE (nmol/L)		**2.57**
≤1.86	1	**($p \leq 0.005$)**
>1.86 to ≤5.01	**7.28 [1.87, 28.36]**	
>5.01	**5.30 [1.65, 17.05]**	
11-HETE (nmol/L)		**2.40**
≤0.39	1	**($p \leq 0.01$)**
>0.39 to ≤0.89	*2.81 [0.86, 9.21]*	
>0.89	**5.18 [1.53, 17.55]**	
17,18-DiHETE (nmol/L)		**0.29**
≤5.59	1	**($p \leq 0.0005$)**
>5.59 to ≤10.10	0.68 [0.18, 2.60]	
>10.10	**0.09 [0.02, 0.34]**	
19,20-HDPA (nmol/L)		**0.23**
≤2.37	1	**($p \leq 0.0001$)**
>2.37 to ≤3.77	0.34 [0.08, 1.47]	
>3.77	**0.05 [0.01, 0.21]**	

[a] Plasma non-esterified oxylipids were separated into tertiles.
[b] Test for exposure was conducted to determine if increases in oxylipid tertiles, compared to the lowest tertile, were associated with being obese. Odds ratio (OR) [95% Confidence Interval (CI)] are displayed.
[c] Test for trend was conducted to determine if increases in oxylipid tertiles were associated with obesity compared to lean individuals. Odds ratio (p-value) are displayed.
[b,c] Polytomous logistic regression was used to regress BMI category on oxylipid tertiles. All data is referenced against the lean BMI category. Both test for trend and test for exposure were adjusted for age and smoking. p-Values bolded if $p \leq 0.05$ or italicized if $p \leq 0.09$ and >0.05.

with 5-HETE concentrations either >1.86 to ≤5.01 nmol/L or >5.01 nmol/L, were over 5 times more likely to be obese than those with 5-HETE concentrations ≤1.86 nmol/L. Results for 11-HETE were similar to 5-HETE, for each category increase in 11-HETE, an individual was 2.4 times more likely to be obese rather than lean. Participants with 11-HETE concentrations >0.89 nmol/L were 5 times more likely to be obese than those with 11-HETE concentrations ≤0.39 nmol/L. Vicinal diols derived from EPA and DHA were inversely associated with obesity. For each category increase in 17,18-DiHETE concentrations, an individual was 0.29 times as likely to be obese rather than lean. Participants with 17,18-DiHETE concentrations >10.10 nmol/L were roughly 0.09 times as likely to be obese than those with 17,18-DiHETE concentrations ≤5.59 nmol/L. Similarly, for each category increase in 19,20-HDPA, an individual was 0.23 times as likely to be obese rather than lean. Patients with 19,20-HDPA concentrations >3.77 nmol/L were 0.05 times as likely to be obese than those with 19,20-HDPA concentrations ≤2.37 nmol/L.

4. Discussion

This study characterized non-esterified plasma PUFA and oxylipid concentrations associated with anthropometrics and serum cytokines. We report that obese participants had higher concentrations of the proinflammatory serum cytokine TNF-α, but TNF-α was not associated with oxylipids. The responses BMI, WC, and serum leptin were highly associated with oxylipids even after FDR p-value correction. BMI, WC, and leptin were positively associated with ω-6 monohydroxy LOX products derived from DGLA and ARA, specifically 15-HETrE, and 5-, 11-, and 15-HETE, respectively. In general, oxylipids derived from the ω-6 PUFA LA were inversely associated with BMI, WC, and leptin, especially epoxides and vicinal diols. In fact, our most significant result is that vicinal diols derived from LA and the ω-3 PUFAs EPA and DHA were inversely associated with BMI, WC, and leptin across several statistical tests, even after normalization to parent PUFA concentrations. C-peptide and total adiponectin were only associated with the EPA-derived vicinal diols 17,18-DiHETE and Total DiHETE after FDR p-value correction, but EPA normalized DiHETE concentrations did not differ across these responses. Non-esterified plasma PUFAs did not differ across any response. Taken together, our results indicate that obesity is associated with specific oxylipids indicative of altered PUFA metabolism through several pathways, rather than attributed to solely altered dietary PUFA intake.

In murine models, a high-fat diet (HFD) induces obesity-associated inflammation [44,45], which mimics chronic low-grade inflammation and metabolic dysregulation in obese humans [46]. In obesity, CYP epoxygenase mRNA is decreased in adipose tissue [20] and sEH is increased in adipose tissue [47] and liver [48]. Increased sEH activity is associated with inflammation (i.e., TNF-α) in several chronic inflammatory diseases, and sEH is a target for pharmacological treatments (reviewed in detail [18]). One would expect that since sEH activity is higher in obesity there would be an increase in vicinal diols. However, here we show that vicinal diols from several PUFAs are inversely associated in the plasma. A majority of research into vicinal diols has focused on those derived from ARA and LA (reviewed in detail [49]). We report that ARA-derived DHETs were only significantly associated with C-peptide when analyzed individually, however, the ratio of 14,15-DHET to 14,15-EET was inversely associated with BMI, WC, leptin, and C-peptide. Unfortunately, 8,9-EET and 11,12-EET had >50% missing values, so these variables were excluded; thus, we could not calculate the ratios of all DHETs to EETs. To our knowledge, we are one of the first groups to report that plasma vicinal diol concentrations are decreased in obese humans. Future research should investigate whether changes in plasma vicinal diol concentrations are related to altered CYP epoxygenase and/or sEH, and if decreases in plasma vicinal diols affect obesity-associated pathology.

The expansion of white adipose tissue induces pathological adipose tissue microenvironments and exacerbates proinflammation, through the recruitment and activation of leukocytes such as macrophages [1]. These leukocytes have LOX enzymes such as 12LOX and 15LOX, which produce oxylipids

involved in the progression or resolution of inflammation [50]. It is well documented that the 12LOX product 12-HETE induces inflammation and leukocyte infiltration into obese adipose tissue [50,51], however, in our study 12-HETE concentrations were not associated with any response. Instead, we observed a linear relationship between concentrations of 15LOX products 15-HETE and 15-HETrE, and BMI, WC, and serum leptin concentrations. 15LOX is elevated in obese adipose tissue [50]. Oxylipids such as 15-HETE are substrates for the synthesis of lipoxins, a class of potent pro-resolving lipid mediators deemed resolvins. Halade et al. [52] reported that excessive ω-6 intake altered LOX expression, decreased resolvin production, and increased TNF-α in aged obese mice. In our study we adjusted statistical models for age, but we did not collect information on dietary intake. To account for the possibility that our results were, in fact, due to altered levels of PUFA substrates (i.e., through dietary intake), we performed regressions on PUFA normalized oxylipid concentrations. None of the responses were significantly associated with 15-HETE concentrations after normalization to ARA levels, suggesting that our observed association between obesity and increased 15-HETE may be related to higher ARA levels in our obese patients. Future studies should perform fluxomic experiments to determine whether increases in plasma 15-HETE concentrations are due to increased metabolism through 15LOX or increased dietary ARA intake.

Additionally, we report that 5- and 11-HETE are elevated in obesity. Increases in 5- and 11-HETE were significantly associated with BMI and WC across all statistical tests, even after normalization to ARA levels. 5-HETE is a 5LOX product and 5LOX is elevated in visceral obese adipose tissue [53]. In a recent randomized controlled trial, supplementation with fish oil did not reduce 5-HETE concentrations in overweight subjects, which suggests that increased 5-HETE is due to altered metabolism rather than PUFA substrate availability [54]. 5-HETE increases TNF-α induced apoptosis of hepatocytes and inhibition of 5LOX decreases hepatic macrophage infiltration of HFD obese mice [55]. It is possible that increases in proinflammatory 5-HETE contribute to pathological changes in obese tissues that promote oxidative stress and increase ROS, which can affect PUFA peroxidation. Interestingly, 11-HETE is a non-enzymatically ARA-derived oxylipid which is a marker of lipid peroxidation [56]. Increased 11-HETE is described as a biomarker ranging from coronary events [57] to cancers [58], however, to our knowledge there is no evidence associating 11-HETE with obesity in humans. We report that individuals with 11-HETE concentrations >0.89 nmol/L were over 5 times more likely to be obese compared to those with ≤0.39 nmol/L. Since obesity is associated with coronary heart disease and cancers, it is unsurprising that 11-HETE may be a biomarker of pathology associated with obesity.

5. Conclusion

In conclusion, our results indicate that BMI, WC, and serum leptin are highly associated with specific oxylipids produced through distinct oxygenating pathways. C-peptide and total adiponectin were only associated with vicinal diols. Our lab has previously published, in this study population, that lipid differences are linked to obesity-associated responses related to excess fat storage in the visceral area (i.e., WC and leptin) [29,59]. In our current study, we report that proinflammatory cytokines are not associated with oxylipids. It is possible that there is no relationship between proinflammatory cytokines and oxylipids in obesity, even though it is well accepted that obesity increases circulating levels of proinflammatory cytokines [1] and these cytokines are involved in pathology that promotes insulin resistance [3]. There is a dearth of research investigating associations between inflammatory cytokines and oxylipids in obesity, making it difficult to compare our results with other studies. For instance, weight loss in obese subjects reduces levels of TNF-α and 5-HETE, however, the authors did not assess the relationship between both TNF-α and 5-HETE [60]. In several inflammatory diseases, such as arthritis [61] and dengue fever [62], TNF-α was associated with oxylipids and markers of lipid peroxidation. Future studies focusing on obesity and oxylipids should also investigate associations between cytokines and oxylipids, so researchers can better understand relationships between circulating proteomic and lipidomic factors. Taken together, our results may have profound implications for novel biomarkers of alerted metabolism in obesity if the significant plasma oxylipids reported in this study directly impact inflammation in obesity, or if plasma concentrations of oxylipids are able to distinguish obese individuals with low-grade inflammation in larger more diverse sample sizes.

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Author Contributions

Concept and design: CAP, LMS, JIF.
Develop of methodology: CZ, LMS.
Statistical analyses: CAP.
Writing, review, and manuscript revision: CAP, LMS, CZ, JIF.
Study supervision: CAP, JIF.

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Conflicts of Interest

None.

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