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ORIGINAL ARTICLE

Clinical Trials and Investigations

Obesity | THE OBESITY SOCIETY WILEY

Omega-6 and omega-3 oxylipins as potential markers of cardiometabolic risk in young adults

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Abstract

Objective: Omega-6 and omega-3 oxylipins are known to play a role in inflammation and cardiometabolic diseases in preclinical models. The associations between plasma levels of omega-6 and omega-3 polyunsaturated fatty acid–derived oxylipins and body composition and cardiometabolic risk factors in young adults were assessed.

Methods: Body composition, brown adipose tissue, traditional serum cardiometabolic risk factors, inflammatory markers, and a panel of 83 oxylipins were analyzed in 133 young adults (age 22.1[SD 2.2] years, 67% women).

Results: Plasma levels of four omega-6 oxylipins (15-HeTrE, 5-HETE, 14,15-EpETrE, and the oxidative stress–derived 8,12-iso-iPF$_{2\alpha}$-VI) correlated positively with adiposity, prevalence of metabolic syndrome, fatty liver index, and homeostatic model assessment of insulin resistance index and lipid parameters. By contrast, the plasma levels of three omega-3 oxylipins (14,15-DiHETE, 17,18-DiHETE, and 19,20-DiHDPA) were negatively correlated with adiposity, prevalence of metabolic syndrome, fatty liver index, homeostatic model assessment of insulin resistance index, and lipid parameters.

The panel of seven oxylipins predicted adiposity better than traditional inflammatory markers such as interferon gamma or tumor necrosis factor-alpha. Pathway analyses revealed that individuals with obesity had higher plasma levels of omega-6 and lower plasma levels of omega-3 oxylipins than normal-weight individuals.

Conclusion: Plasma levels of seven omega-6 and omega-3 oxylipins may have utility as early markers of cardiometabolic risk in young adults.

INTRODUCTION

The global prevalence of obesity and cardiometabolic diseases has increased progressively in the past decades to reach pandemic proportions (1). A major risk factor for cardiometabolic diseases is the so-called metabolic syndrome, which is defined as a constellation of metabolic disorders, including insulin resistance and impaired glucose tolerance, atherogenic dyslipidemia (high triglycerides [TG], total cholesterol [TC], and low-density lipoprotein cholesterol [LDL-C], and low high-density lipoprotein cholesterol [HDL-C]), and a pro-inflammatory state accompanied by excess visceral adipose tissue (VAT) mass (2).

Omega-6– and omega-3–derived lipid mediators have a prominent role in inflammation and cardiometabolic diseases as they possess pro- and anti-inflammatory properties (3,4). Omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids (i.e., they should be obtained from the diet) because they cannot be synthesized endogenously in humans (5). Upon dietary ingestion, circulating omega-6 and omega-3 PUFAs are used by almost all tissues and are oxidized by several enzymatic steps involving cyclooxygenase, lipoxygenase, or cytochrome P450 enzymes (6). These enzymatic reactions facilitate the conversion of PUFAs to oxylipins and other bioactive lipids, which are major mediators of the pro- and anti-inflammatory effects of PUFAs on human metabolism (6).

Curiously, circulating oxylipin levels might not necessarily mimic dietary PUFA intake, because oxylipin production depends on enzyme preference for specific PUFAs (6). Oxylipins are reported to be produced by various tissues, including white adipose tissue, liver, kidney, and ileum (6). Additionally, brown adipose tissue (BAT), a metabolically active thermogenic tissue present in adult humans (7-9), has been suggested to secrete omega-6 (e.g., 5-HETE, 5,6-EpETrE, 12,13-DiHOME) and omega-3 (e.g., 12-HEPE) oxylipins (10-13).

Study Importance

What is already known?

▶ Omega-6 and omega-3 oxylipins are breakdown products of polyunsaturated fatty acids, which play a role in inflammation and cardiometabolic diseases. The *fat-1* transgenic mouse, which transforms omega-6 into omega-3 polyunsaturated fatty acids, is protected against cardiometabolic diseases and shows lower levels of omega-6 oxylipins, including 15-HeTrE, 5-HETE, and 14,15-EpETrE, and higher levels of omega-3 oxylipins, including 14,15-DiHETE, 17,18-DiHETE, and 19,20-DiHDPA.

What does this study add?

▶ We show four omega-6 oxylipins and three omega-3 oxylipins strongly related to body composition and cardiometabolic risk factors in young adults. These seven oxylipins are better predictors of adiposity than traditional pro- and anti-inflammatory markers and are identical to those previously described in the *fat-1* mice, suggesting a causal relationship. This set of oxylipins may be considered to diagnose cardiometabolic risk in young adults.

How might these results change the direction of research or the focus of clinical practice?

▶ Plasma levels of seven omega-6 and omega-3 oxylipins may have utility as early markers of cardiometabolic risk in young adults.

Although omega-6 oxylipins have proinflammatory, vasoconstrictor, and proliferative functions, all of which are involved in the progression of obesity and cardiometabolic diseases (6,14), omega-3 oxylipins have the opposite effects (6,14). Notably, plasma levels of the omega-6 oxylipins 9,10-EpODE and 9,10-EpOME are higher in women with type 2 diabetes than in nondiabetic peers (15). Likewise, plasma levels of omega-6 (8,9-DiHETrE) and omega-3 (5-HEPE and 10,11-DiHDPE) oxylipins are higher in men with hyperlipidemia than in normolipidemic men (16). These studies support a relationship between omega-6 oxylipins and cardiometabolic disorders; however, the relationship between both omega-6 and -3 oxylipins and cardiometabolic risk factors in young, relatively healthy individuals has not yet been established.

Here we investigated the associations of plasma levels of omega-6 and -3 PUFA–derived oxylipins with body composition and cardiometabolic risk factors in a well-phenotyped cohort of young adults.

METHODS

See online Supporting Information for an extensive description of all methods.

Study design and participants

A total of 136 participants, including 91 women and 45 men (age 22.1 [SD 2.2] years), were included in the present study (ACTIBATE; ClinicalTrials.gov ID: NCT02365129) (17). The ACTIBATE study encompassed multiple evaluation waves involving groups of 16 to 24 individuals every 2 to 3 weeks in October, November, and December of 2015 and 2016. The study was approved by the Ethics Committee on Human Research of the University of Granada (n° 924) and the *Servicio Andaluz de Salud* and was in accordance with the principles of the latest version of the Declaration of Helsinki. Written informed consent was provided from all participants before their enrollment. Inclusion criteria were the following: (1) nonsmoking, (2) sedentary (i.e., <20 minutes of moderate-to-vigorous physical activity on <3 days per week), (3) no acute or chronic illness, (4) no medication that might interfere with the assessments, and (5) not pregnant. Participants were requested to be rested, to abstain from moderate-to-vigorous physical activity, to use bus or car transportation, to refrain from stimulants and/or alcohol on the days of the measurements, and to have not performed any moderate exercise in the previous 24 hours or vigorous exercise in the previous 48 hours. A total of 133 participants were included in the main analyses; 3 participants were excluded because of blood collection issues.

Anthropometric and body composition measurements

Body weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm, using a model 799 scale and a stadiometer,

respectively (both from Seca), without shoes and with light clothing. BMI was calculated from weight and height (kilograms/meters squared). Waist circumference was measured at the minimum perimeter, at the end of a normal expiration, with the arms relaxed on both sides of the body. When the minimum perimeter could not be detected, measurements were taken just above the umbilicus, in a horizontal plane. Waist circumference was measured twice with a plastic tape measure; the two measures were averaged for further analyses.

Lean, fat, and VAT masses were measured by dual-energy x-ray absorptiometry using a Discovery Wi device (Hologic Inc.) equipped with analysis software (APEX version 4.0.2). Lean and fat mass indices were expressed as kilograms/meters squared; fat mass was also expressed as a percentage of body weight. Individuals were categorized according to their BMI as having normal weight (BMI <25.0 kg/m^2), overweight (BMI ≥25.0 kg/m^2 and <30.0 kg/m^2), or obesity (BMI ≥30.0 kg/m^2) (18).

Assessment of ^{18}F-fluorodeoxyglucose uptake by BAT

Briefly, the shivering threshold of each participant was determined following a personalized cooling protocol (19). Then 48 to 72 hours after the shivering threshold determination, participants were exposed to a 2-hour personalized cooling procedure at 3.8°C above their individual shivering threshold. After 1 hour of cold exposure, a bolus of ~185 MBq of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) was intravenously injected, and the water temperature was increased by 1°C. If participants reported shivering, the water temperature was further increased by 1°C. After 2 hours of cold exposure, a static positron emission tomography/computed tomography (PET/CT; Siemens Biograph 16 PET-CT) scan was performed. CT acquisition was performed using a peak of 120 kV and PET acquisition with a scan time of 6 minutes per bed position. PET/CT images were obtained from the atlas (i.e., cervical vertebra 1) to approximately the midchest. BAT-related outcomes were calculated as described (20-22).

Blood sample collection and determination of cardiometabolic risk factors

Blood was collected after an overnight (10 hours) fast, between 8:00 and 9:00 am, and at room temperature (20°C to 24°C). Blood was drawn from the antecubital vein and was immediately centrifuged to obtain serum (obtained with Vacutainer SST II Advance tubes, VWR International, LLC) and plasma (obtained with Vacutainer Hemogard tubes, containing the K2 potassium salt of ETDA as anticoagulant). Samples were aliquoted and stored at −80°C.

Traditional cardiometabolic risk factors and inflammatory markers were measured in serum (glucose, insulin, cortisol, TC, HDL-C, TG, apolipoproteins A and B, glutamic pyruvic transaminase [GTP], gamma-glutamyl transferase [GGT], alkaline phosphatase [ALP],

C-reactive protein, complement C3, complement C4, leptin, adiponectin, interleukin [IL]-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-17a, interferon gamma [IFNɣ], and tumor necrosis factor-alpha [TNFα]).

Metabolic syndrome prevalence was calculated following the National Cholesterol Education Program Adult Treatment Panel III (ATP III) (23) and International Diabetes Federation (IDF) classifications (24). Insulin sensitivity was estimated via the homeostatic model assessment of insulin resistance index (HOMA-IR) (25), and the fatty liver index was determined as a proxy of hepatic steatosis (26).

Determination of plasma oxylipins

Oxylipins were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (27). The LC-MS/MS protocol enabled the relative quantitation of 83 oxylipins from the conversion of the omega-6 PUFAs linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), and arachidonic acid (AA), as well as the omega-3 PUFAs α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The oxylipins that can be detected by this method are listed in Supporting Information Table S1. Of the 50 oxylipins detected, 40 showed a low analytical variability with relative standard in quality control (QC_{RSD}) ≤ 15%, and 10 showed a moderate variability between 15% < QC_{RSD} ≤ 40% (Supporting Information Table S1).

Statistical analyses

The baseline characteristics and outcomes of the study participants were expressed as mean (SD). The normal distribution assumption was tested using the Shapiro–Wilk test, visual histograms, and Q–Q plots. Non-normally distributed variables (traditional cardiometabolic and inflammatory markers and oxylipins) were log_{10}-transformed before further analysis. We performed all the analyses divided by men and women, and similar patterns were observed. Moreover, we studied whether significant interactions were presented between the plasma oxylipin levels and body composition and cardiometabolic risk parameters using linear regression models, and no sex interactions were observed (all $p \geq 0.05$). Thus, data of men and women were analyzed together.

We conducted Pearson correlation analyses to examine the relationship between plasma levels of oxylipins and body composition and cardiometabolic risk parameters. All p values were corrected by the two-stage step-up procedure of Benjamini, Krieger, and Yekutieli for multiple comparisons by controlling the false discovery rate (FDR) (28). Plasma omega-6 and omega-3 oxylipins were both weakly correlated with PUFA, fish, and shellfish intake (data not shown). We therefore performed partial correlations of plasma levels of oxylipins with body composition and cardiometabolic risk parameters adjusting for dietary energy, PUFA, fish, and shellfish intake. Pearson correlation analyses were also carried out between plasma levels of oxylipins and traditional inflammatory parameters. As secondary analyses, we examined the relationship between

body composition and cardiometabolic risk factors. All correlation analyses and plots were designed using the "corrplot" package in R software version 3.6.0 (R Foundation for Statistical Computing). Forward stepwise regression was performed using fat mass percentage and VAT mass as dependent outcomes in separate models. The measured oxylipins persisting after FDR correction (i.e., 15-HETrE, 5-HETE, 14,15-EpETrE, 8,12-iso-iPF$_{2α}$-VI, 14,15-DiHETE, 17,18-DiHETE, and 19,20-DiHDPA), together with the traditional cardiometabolic risk factors HOMA-IR, GTP, GGT, ALP, TC, HDL-C, TG, cortisol, adiponectin, and leptin, were introduced as predictors using a forward stepwise procedure, which introduces the predicting components step-by-step into the model (if $p < 0.05$) according to the strength of their association with the dependent outcome. We repeated the same analyses including plasma levels of oxylipins and traditional pro- and anti-inflammatory markers as predictors of fat mass percentage and VAT mass. The forward stepwise regression was performed using the SPSS Statistics version 25.0 (IBM Corp.).

Differences in plasma levels of oxylipins across BMI categories (normal weight, overweight, and obesity) were assessed with metabolic pathway analyses using Cytoscape software version 3.7.0 (Cytoscape Consortium) (29). We calculated a fold change for each oxylipin as the ratio between the oxylipin level in individuals with obesity divided by the oxylipin level in normal-weight individuals, as well as between obesity and overweight and between overweight and normal weight. Independent t test analyses were performed for between-group comparisons. The level of significance was set at $p < 0.05$, using SPSS version 25.0.

RESULTS

The descriptive characteristics of the participants are presented in Table 1. A total of 50 of the 83 detectable PUFA-derived omega-6 ($n = 30$) and omega-3 ($n = 20$) oxylipins, including their precursors, were detected in fasting plasma samples (Supporting Information Table S1). Baseline plasma levels of each oxylipin measured in the participants are listed in Supporting Information Table S2.

Plasma levels of oxylipins are related to body composition parameters

At the first step, we investigated the relationship between the plasma levels of omega-6 and omega-3 oxylipins and body composition parameters. A total of 12 of the 30 detected omega-6 oxylipins correlated positively with fat mass percentage (all $r \geq 0.18$; $p < 0.05$; Figure 1A), whereas 3 of the 20 detected omega-3 oxylipins correlated negatively with fat mass percentage (all $r \leq -0.29$; $p < 0.05$; Figure 1B). Similar correlation coefficients were obtained between the aforementioned oxylipins and fat mass index or VAT mass (all $r \leq -0.23$; $p < 0.05$; Figure 1A,B). After FDR correction, four omega-6 oxylipins (15-HETrE, 5-HETE, 14,15-EpETrE, and 8,12-iso-iPF$_{2α}$-VI)

TABLE 1 Characteristics of the participants

	n	Total	n	Men	n	Women
Age (y)	136	22.1 (2.2)	45	22.3 (2.3)	91	21.9 (2.2)
Body composition						
BMI (kg/m^2)	136	24.9 (4.6)	45	26.8 (5.5)	91	23.9 (3.7)
Lean body mass (kg)	136	41.8 (9.7)	45	52.8 (7.2)	91	36.3 (5.0)
Lean mass index (kg/m^2)	136	14.7 (2.4)	45	17.2 (2.1)	91	13.5 (1.4)
Fat body mass (kg)	136	24.7 (8.8)	45	24.8 (11.0)	91	24.6 (7.5)
Fat mass (%)	136	35.5 (7.6)	45	29.7 (7.6)	91	38.3 (5.9)
Fat mass index (kg/m^2)	136	8.8 (3.0)	45	8.1 (3.6)	91	9.1 (2.7)
Visceral adipose tissue mass (g)	136	336.4 (174.1)	45	417.9 (175.9)	91	296.1 (159.2)
Waist circumference (cm)	130	81.0 (4.6)	43	89.9 (15.2)	87	76.5 (10.5)
BAT						
BAT volume (mL)	131	68.5 (57.4)	42	78.9 (66.0)	89	63.6 (52.6)
BAT metabolic activity	131	332.9 (328.7)	42	326.8 (327.8)	89	335.8 (331.0)
BAT SUVmean	131	3.7 (1.9)	42	3.2 (1.3)	89	4.0 (2.1)
BAT SUVpeak	131	11.1 (8.2)	42	9.9 (7.3)	89	11.6 (8.6)
BAT SUVmax	131	12.2 (9.0)	42	10.8 (8.1)	89	12.8 (9.4)
Cardiometabolic risk factors						
Metabolic syndrome ATP III	128	0.5 (0.9)	42	1.0 (1.3)	86	0.2 (0.5)
Metabolic syndrome IDF	128	0.7 (1.1)	42	1.1 (1.5)	86	0.5 (0.7)
Fatty liver index	132	20.4 (25.0)	43	36.9 (32.0)	89	12.5 (15.7)
GTP (IU/L)	131	19.0 (17.5)	43	28.4 (26.8)	88	14.4 (6.7)
GGT (IU/L)	131	19.8 (20.0)	43	29.9 (29.8)	88	14.9 (9.9)
ALP (IU/L)	132	71.3 (18.5)	43	79.3 (19.4)	89	67.5 (16.9)
C-reactive protein (mg/L)	132	2.4 (3.4)	43	2.1 (2.3)	89	2.5 (3.8)
C3 (mg/dL)	132	137.4 (23.8)	43	143.0 (26.2)	89	134.7 (22.2)
C4 (mg/dL)	132	28.7 (8.8)	43	30.3 (9.9)	89	27.9 (8.1)
Glucose (mg/dL)	132	87.6 (6.6)	43	88.9 (7.4)	89	87.0 (6.1)
Insulin (µIU/mL)	132	8.3 (4.9)	43	9.1 (6.4)	89	8.0 (4.0)
HOMA-IR	132	1.8 (1.2)	43	2.1 (1.6)	89	1.7 (1.0)
Insulin glucose ratio	132	14.1 (7.0)	43	14.8 (8.8)	89	13.8 (6.0)
Total cholesterol (mg/dL)	132	165.1 (32.2)	43	160.1 (30.9)	89	167.6 (32.7)
HDL-C (mg/dL)	132	52.8 (11.0)	43	46.0 (7.4)	89	56.0 (11.0)
LDL-C (mg/dL)	132	96.0 (25.3)	43	96.5 (26.2)	89	95.8 (25.0)
APOA1 (mg/dL)	113	144.7 (27.5)	37	130.0 (16.8)	76	151.9 (28.9)
APOB (mg/dL)	113	69.7 (19.9)	37	72.7 (24.4)	76	68.3 (17.3)
Triglycerides (mg/dL)	132	82.5 (44.6)	43	88.2 (47.2)	89	79.7 (43.2)
Leptin (µg/L)	129	6.2 (4.4)	42	4.4 (4.0)	87	7.1 (4.3)
Adiponectin (mg/L)	127	11.4 (7.9)	42	7.7 (5.2)	85	13.3 (8.3)
Systolic blood pressure (mmHg)	134	116.7 (11.6)	44	125.3 (10.9)	90	112.5 (9.5)
Diastolic blood pressure (mmHg)	134	70.9 (7.6)	44	72.2 (9.2)	90	70.3 (6.7)
Inflammatory parameters						
IL-2 (pg/mL)	109	2.4 (1.4)	37	2.0 (1.3)	72	2.6 (1.5)
IL-4 (pg/mL)	109	13.1 (9.7)	37	11.7 (9.5)	72	13.8 (9.7)
IL-6 (pg/mL)	109	1.6 (1.6)	37	1.6 (1.8)	72	1.6 (1.5)
IL-7 (pg/mL)	109	4.0 (2.8)	37	3.3 (2.2)	72	4.4 (3.0)

(Continues)

TABLE 1 (Continued)

	n	Total	n	Men	n	Women
IL-8 (pg/mL)	109	1.6 (0.8)	37	1.5 (0.9)	72	1.6 (0.8)
IL-10 (pg/mL)	109	2.9 (3.6)	37	2.3 (2.1)	72	3.2 (4.1)
IFN-γ (pg/mL)	109	12.9 (5.4)	37	11.5 (5.5)	72	13.5 (5.3)
TNFα (pg/mL)	109	1.8 (1.1)	37	1.5 (0.7)	72	1.9 (1.2)

Data are presented as mean (SD).

Abbreviations: ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; ATP III, Adult Treatment Panel III; BAT, brown adipose tissue; C3, complement C3; C4, complement C4; GGT, gamma-glutamyl transferase; GTP, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance index; IDF, International Diabetes Federation; IFN-γ, interferon gamma; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; SUV, standardized uptake value; TNFα, tumor necrosis factor-alpha.
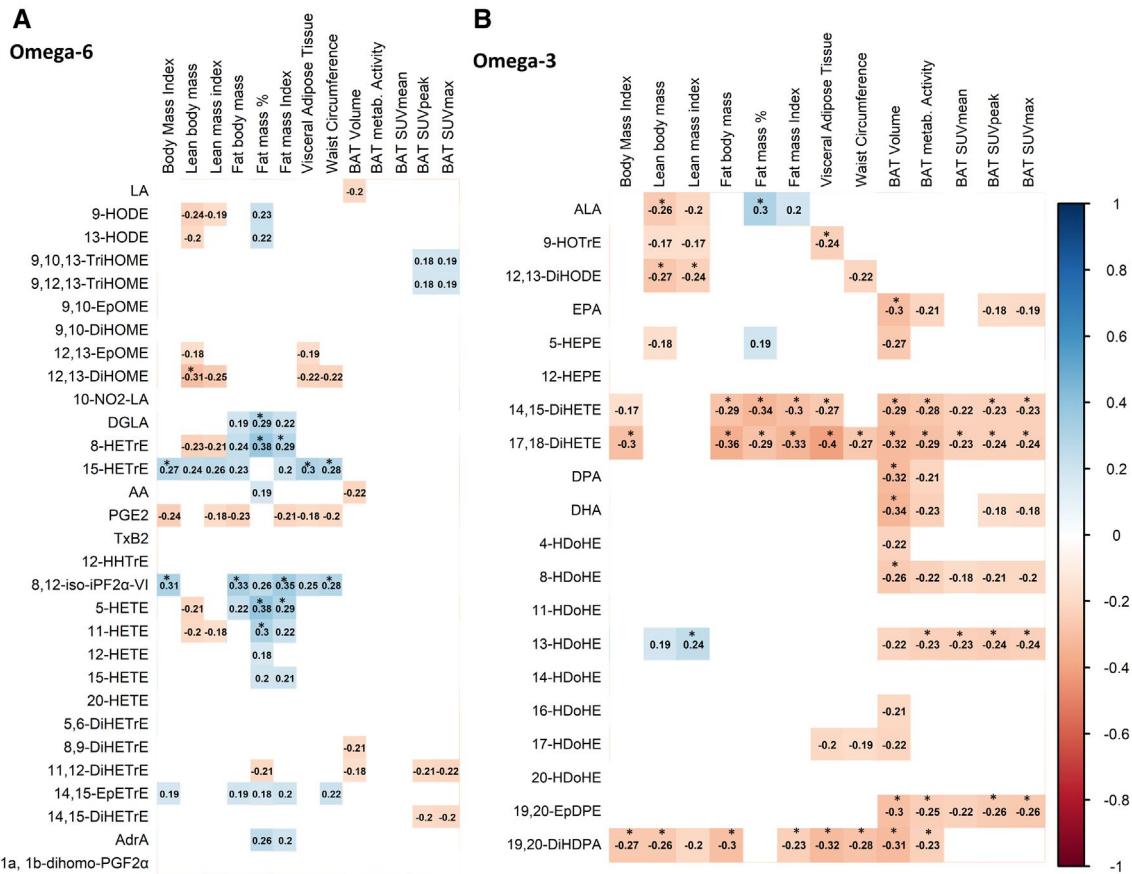
FIGURE 1 Pearson correlation analyses between plasma levels of oxylipins (omega-6 and omega-3) with body composition and brown adipose tissue (BAT) parameters in young adults ($n = 133$). Every box represents a significant correlation coefficient (all $p < 0.05$), whereas empty spaces represent no significant correlations. Blue and red boxes indicate positive and negative correlations, respectively. All oxylipin outcomes were $\log_{10}$-transformed prior to data analysis. *Significant after false discovery rate corrections. SUV, standardized uptake value. The abbreviations used for each oxylipin are detailed in Supporting Information Table S1

and three omega-3 oxylipins (14,15-DIHETE, 17,18-DIHETE, and 19,20-DiHDPA) remained significantly correlated with adiposity parameters (fat mass percentage, fat mass index, and VAT mass; all $p < 0.05$; Figure 1A,B).

Given the clear link between plasma oxylipins and dietary PUFA intake (30), we next conducted partial correlation analysis adjusting for dietary energy, PUFA, fish, and shellfish intake as cofounders. We found that the significant correlations between the set of four omega-6 and three omega-3 oxylipins with adiposity parameters

remained significant (Supporting Information Figure S1), suggesting that these correlations are not affected by dietary energy, PUFA, fish, and shellfish intake.

Because BAT can be a source of oxylipins (10-13) and has been linked to a healthier cardiometabolic profile in humans (31), we explored whether plasma oxylipin levels correlated with BAT volume and activity. After FDR correction, plasma levels of the omega-3 oxylipins 14,15-DiHETE, 17,18-DiHETE, 19,20-EpDPE, and 19,20-DiHDPA correlated negatively with BAT volume and activity

(BAT metabolic activity, SUVmean, SUVpeak, and SUVmax; all $r \leq$ −0.23; $p < 0.05$; Figure 1B). By contrast, no significant correlations were found between plasma omega-6 oxylipins and BAT volume or activity (Figure 1A). Of note, the associations remained unaltered when dietary intake (i.e., energy, PUFA, fish, and shellfish intake; Supporting Information Figure S1) and the seasonal variation were included as cofounders, the latter of which is known to affect the determination of BAT volume and activity in humans (31,32) (data not shown).

Plasma levels of oxylipins are related to cardiometabolic risk factors

We next examined whether plasma oxylipin levels were related to traditional cardiometabolic risk factors assessed in serum. Notably, the set of four omega-6 oxylipins that correlated positively with adiposity parameters (15-HeTrE, 5-HETE, 14,15-EpETrE, and 8,12-iso-iPF$_{2\alpha}$-VI) also correlated positively with an adverse cardiometabolic profile (i.e., higher prevalence of metabolic syndrome,

higher fatty liver index, serum glucose, and serum lipid parameters [TC or TG]; $r \geq 0.18$; $p < 0.05$; Figure 2A). By contrast, the set of three omega-3 oxylipins that correlated negatively with adiposity parameters (14,15-DIHETE, 17,18-DIHETE, and 19,20-DiHDPA) correlated with a favorable cardiometabolic profile, specifically lower prevalence of metabolic syndrome, fatty liver index, serum glucose, and serum lipid parameters (TC or TG) ($p < 0.05$; Figure 2B). The correlations remained significant after adjusting for dietary energy, PUFA, fish, and shellfish intake (Supporting Information Figure S2), although they were not significant after correcting for FDR.

Based on the pro- and anti-inflammatory effects of the omega-6 and omega-3 oxylipins, respectively, we expected to observe significant correlations with traditional pro- and anti-inflammatory markers (IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-17a, IFNγ, and TNFα). We found that plasma levels of three of the four omega-6 oxylipins that correlated positively with adiposity parameters and an adverse cardiometabolic profile (15-HeTrE, 5-HETE, and 8,12-iso-iPF$_{2\alpha}$-VI) also correlated positively with the proinflammatory markers IL-17, IFNγ, and TNFα and negatively with the anti-inflammatory marker IL-4. Surprisingly, we found that the plasma levels of the three
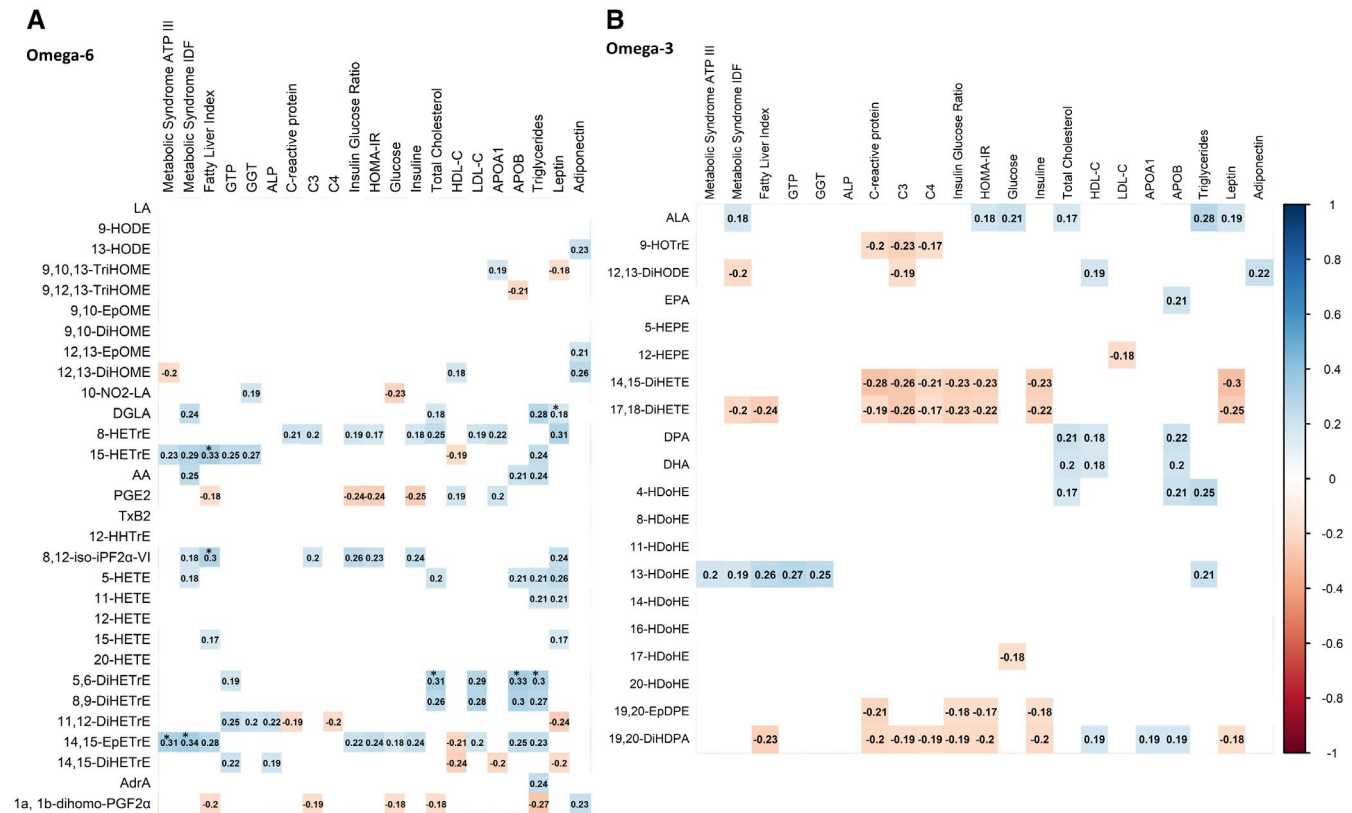

FIGURE 2 Pearson correlation analyses between plasma levels of oxylipins (omega-6 and omega-3) with cardiometabolic risk factors in young adults ($n = 133$). Every box represents a significant correlation coefficient (all $p < 0.05$), whereas empty spaces represent no significant correlations. Blue and red boxes indicate positive and negative correlations, respectively. All oxylipin outcomes were log$_{10}$-transformed prior to data analysis. *Significant after false discovery rate corrections. ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; ATP III, National Cholesterol Education Program Adult Treatment Panel III; GGT, gamma-glutamyl transferase; GTP, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance index; IDF, International Diabetes Federation; LDL-C, low-density lipoprotein cholesterol. The abbreviations used for each oxylipin are detailed in Supporting Information Table S1

omega-3 oxylipins that correlated negatively with adiposity parameters (14,15-DIHETE, 17,18-DIHETE, and 19,20-DiHDPA) did not correlate with traditional pro- and anti-inflammatory makers (all $r \geq 0.19$; $p < 0.05$; Supporting Information Figure S3B).

Given these findings, we used stepwise linear regression models to assess whether plasma levels of oxylipins were better predictors of adiposity than traditional cardiometabolic risk factors or established pro- and anti-inflammatory markers. We selected the four omega-6 and 3 omega-3 oxylipins that were related to adiposity and cardiometabolic risk parameters (Figure 1A,B, Figure 2A,B, and Figure 3). The panel of seven oxylipins was included together with classical cardiometabolic risk factors as independent outcomes, and fat mass percentage or VAT mass was included as dependent outcomes in separate models (Supporting Information Table S3). We repeated these analyses including the panel of oxylipins together with established pro- and anti-inflammatory markers as independent outcomes (Table 2). Overall, the traditional cardiometabolic risk factors, principally leptin and HOMA-IR, were found to be better predictors of adiposity (fat mass percentage and VAT mass) than the panel of seven oxylipins (Supporting Information Table S3). However, the plasma levels of the omega-6 oxylipins 5-HETE and 15-HETrE and the plasma levels of 14,15-DiHETE and 17,18-DiHETE (omega-3

oxylipins) were better predictors of adiposity than established pro- and anti-inflammatory markers (e.g., IL-6, TNFα) (Table 2), predicting up to 27.2% and 22.9% of the explained variance of the fat mass percentage and VAT mass, respectively (Table 2). The stepwise linear regression models were repeated with the inclusion of all oxylipins, resulting in similar results to those observed with the panel of seven oxylipins (data not shown).

Individuals with obesity have higher plasma levels of omega-6 and lower plasma levels of omega-3 oxylipins than normal-weight individuals

To further investigate the relationship between plasma levels of oxylipins and adiposity and cardiometabolic risk factors, we performed pathway interaction network analyses comparing plasma levels of oxylipins between individuals with obesity and normal-weight individuals (Figure 4). We found that the metabolic pathway related to omega-6 oxylipins, which includes DGLA, 8-HETrE, 15-HETrE, $8,12$-iso-iPF$_{2\alpha}$-VI, 14,15-EpETrE, and 15-HETE, was higher in individuals with obesity ($n = 20$) than in normal-weight ($n = 82$) individuals (fold change 1.26 to 1.47; $p \leq 0.005$; Figure 4A). By contrast,
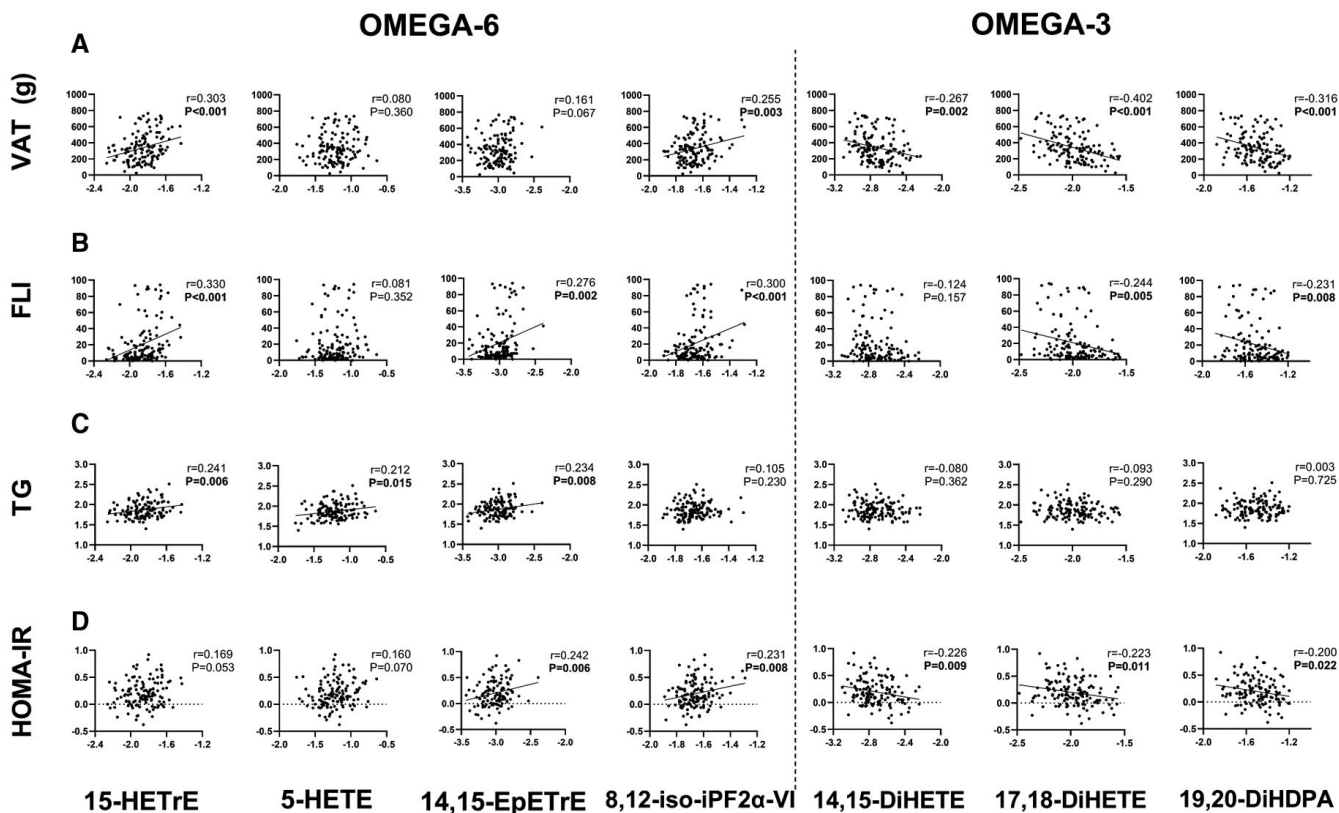

FIGURE 3 Scatterplots between plasma levels of selected oxylipins (15-HETrE, 5-HETE, 14,15-EpETrE, 8,12-iso-iPF$_{2\alpha}$-VI, 14,15-DiHETE, 17,18-DiHETE, and 19,20-DiHDPA) and VAT, FLI, TG, and HOMA-IR. Pearson correlations were performed. All oxylipins values and triglycerides were log$_{10}$-transformed. FLI, fatty liver index; HOMA-IR, homeostatic model assessment of insulin resistance index; TG, triglycerides; VAT, visceral adipose tissue. The abbreviations used for each oxylipin are detailed in Supporting Information Table S1

TABLE 2 Forward stepwise regression models assessing the independent association of the predictors (selected oxylipins and traditional pro- and anti-inflammatory markers) with fat mass percentage and visceral adipose tissue mass as dependent outcomes in separate models in young adults

	β	B	95% CI	p (coefficients)	R^2	R^2 change	F change	p (model)
Fat mass percentage (%)								
Step 1					0.167	–	22.457	**<0.001**
14,15-DiHETE	−0.409	−16.588	−23.523, −9.652	**<0.001**				
Step 2					0.272	0.105	16.078	**<0.001**
14,15-DiHETE	−0.390	−15.831	−22.353, −9.309	**<0.001**				
5-HETE	0.325	11.815	5.976, 17.654	**<0.001**				
Step 3					0.308	0.035	5.641	**<0.001**
14,15-DiHETE	−0.398	−16.148	−22.545, −9.752	**<0.001**				
5-HETE	0.311	11.284	5.545, 17.022	**<0.001**				
IL-8	0.189	6.048	1.001, 11.095	**0.019**				
Visceral adipose tissue mass (g)								
Step 1					0.126	–	16.211	**<0.001**
17,18-DiHETE	−0.356	−323.799	−483.141, −164.456	**<0.001**				
Step 2					0.229	0.103	14.846	**<0.001**
17,18-DiHETE	−0.333	−303.105	−453.818, −152.392	**<0.001**				
15-HETrE	0.322	336.505	163.444, 509.567	**<0.001**				

Selected oxylipins: 15-HETrE, 5-HETE, 14,15-EpETrE, 8,12-iso-iPF$_{2\alpha}$-VI, 14,15-DiHETE, 17,18-DiHETE, and 19,20-DiHDPA; and traditional inflammatory markers: IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-17a, IFNγ, and TNFα.

Abbreviations: β, standardized regression coefficient; B, unstandardized regression coefficient; IL-8, interleukin-8; R^2, adjusted coefficient of determination, expressing the percent variability of the dependent variable explained by each model; R^2 change, additional percent variability explained by the model due to the inclusion of the new term.
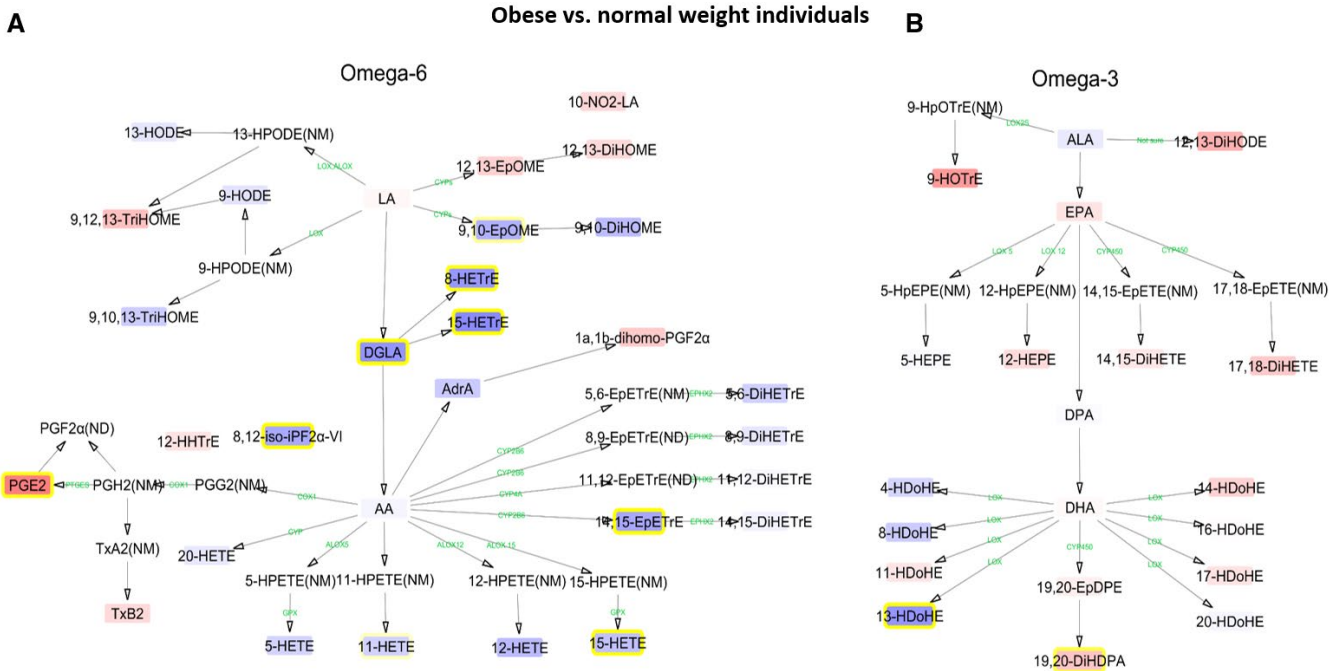

FIGURE 4 Interaction network pathway analyses of plasma levels of omega-6 and omega-3 polyunsaturated fatty acids in individuals with obesity versus normal-weight individuals. The networks depict the differences of individual (A) omega-6 and (B) omega-3 lipid mediators in obesity compared with normal-weight individuals. Blue boxes with yellow frame and red boxes with yellow frame indicate higher and lower levels, respectively, in both populations. Blue and red boxes without yellow frame are oxylipins without statistical significance. Enzymes involved in the metabolic pathway are represented in green. All oxylipin outcomes were log$_{10}$-transformed prior to data analysis. Oxylipins without boxes were not detected (ND) or not measured (NM). The abbreviations used for each oxylipin are detailed in Supporting Information Table S1

the omega-3 metabolic pathway tended to be lower in people with obesity, although only the plasma levels of 19,20-DiHDPA reached the significance threshold (fold change 0.81; $p = 0.014$; Figure 4B).

DISCUSSION

We identified seven oxylipins as potential subclinical markers of adverse cardiometabolic health in young adults. Using targeted metabolomics, we found that the plasma levels of 15-HeTrE, 5-HETE, 14,15-EpETrE, and 8,12-iso-iPF$_{2\alpha}$-VI omega-6 oxylipins were positively correlated with adiposity, the prevalence of metabolic syndrome, fatty liver index, HOMA-IR, and lipid parameters, whereas the plasma levels of 14,15-DiHETE, 17,18-DiHETE, and 19,20-DiHDPA omega-3 oxylipins were negatively correlated with adiposity, the prevalence of metabolic syndrome, fatty liver index, and HOMA-IR and lipid parameters. The panel of seven oxylipins was a better predictor of adiposity than traditional pro- and anti-inflammatory markers (e.g., IFNɣ, TNFα). We also found that obesity was linked to higher plasma levels of omega-6 and lower plasma levels of omega-3 oxylipins.

Role of oxylipins in the development of adiposity and cardiometabolic diseases

Our data show that the plasma levels of a panel of seven oxylipins may be used as an early marker of cardiometabolic risk in young adults. Evidence in support of this notion comes from preclinical studies using the *fat-1* transgenic mouse, which expresses an omega-3 desaturase that transforms omega-6 into omega-3 PUFAs and overcomes the enzymatic competition between omega-6 and omega-3 (33). This mouse model is protected against obesity and cardiometabolic diseases such as insulin resistance, liver steatosis, metabolic syndrome, and chronic inflammation when exposed to high-fat diet (34) and it shows lower plasma levels of omega-6 oxylipins, including 15-HeTrE, 5-HETE, and 14,15-EpETrE, and higher plasma levels of omega-3 oxylipins, including 14,15-DiHETE, 17,18-DiHETE, and 19,20-DiHDPA (35). Accordingly, the set of oxylipins described in *fat-1* mice is identical to the panel of oxylipins found in our human study, supporting their role as important players in metabolic homeostasis. The authors of the latter study suggested that this oxylipin panel might be used as a potential marker of the cardiometabolic risk status of mice (35), which is confirmed in the present human cohort. Indeed, the aforementioned preclinical study supports the notion that the balance between omega-6 and omega-3 oxylipins is the likely main mechanism that explains the protective phenotype, adding weight to the idea that the observational findings in the present study may be due to causal relationships. It is worth highlighting that 8,12-iso-iPF$_{2\alpha}$-VI is a potential marker of cardiometabolic health in humans; this omega-6 oxylipin 8,12-iso-iPF$_{2\alpha}$-VI is produced by the nonenzymatic oxidation of membrane-linked AA and is a marker of free radical attack/oxidative stress (36). Evidence for its role in metabolic disorders was recently supplied by the observation that people with type 2 diabetes have higher levels of 8,12-iso-iPF$_{2\alpha}$-VI than those

without (37), which is consistent with our findings. Accordingly, we found that 8,12-iso-iPF$_{2\alpha}$-VI was associated with increased indices of adiposity and with cardiovascular markers, which are linked to permanent prooxidative stress, ultimately leading to systemic/local damage and clinical cardiovascular disease.

Role of oxylipins as potential markers of the overall inflammatory status

Our study establishes a panel of seven oxylipins as good markers of subclinical inflammation even before observed changes to proinflammatory cytokines, which are associated with increased adipose tissue accretion. This panel was related to adiposity and showed a coherent relationship with proinflammatory and anti-inflammatory cytokines, before the traditional inflammatory markers, such as IFNɣ or TNFα, emerged as indicators of a chronic inflammatory environment. It is well known that lipoxins, which are formed from omega-6 PUFA breakdown, include several proinflammatory lipid mediators such as prostaglandins, thromboxane, and leukotrienes. By contrast, breakdown of omega-3 PUFAs produces specialized proresolving lipid mediators such as resolvins, maresins, and protectins, which regulate anti-inflammatory processes and allow for reestablishment of tissue homeostasis. Indeed, oxylipins influence cytokine production and release, the coagulation cascade, and leukocyte chemotaxis (38,39), supporting their role as early contributors to the low-grade systemic inflammatory status in young adults.

Omega-3 oxylipins are related to low ^{18}F-FDG uptake by BAT

We observed that plasma levels of the omega-3 oxylipins 14,15-DiHETE, 17,18-DiHETE, 19,20-EpDPE, and 19,20-DiHDPA were negatively correlated with BAT volume and activity, whereas omega-6 oxylipins showed no association with BAT-related outcomes. BAT is a metabolic tissue with thermogenic and cardiometabolic protective functions (31). Therefore, our findings are of clinical interest because of the secretory function of BAT, which might contribute to the pool of circulating oxylipins (10-13), although it cannot be confirmed in the current study. At first glance, these findings would appear at odds with earlier studies showing that BAT has a role in the regulation of the systemic pool of omega-3 and -6 oxylipins after cold exposure, increasing the levels of the former and decreasing the levels of the latter (10,11,40). It should be noted, however, that previous studies measured oxylipin levels before and after either a cold exposure or a single bout of exercise in small cohorts (all $n \leq 40$) (10,11,12,13,41), and none of them measured fasting levels of oxylipins, as was done here. Therefore, comparisons between the present findings and those of earlier studies must be made carefully. Regarding the biological significance of the set of negative correlations we observed, it is known that the heart consumes both glucose and fatty acids and that it can switch between

glycolytic or lipolytic-based pathways depending on the availability of substrates (42). BAT also consumes both glucose and fatty acids (43), but it is not yet known whether it has the ability to switch between oxidative and glycolytic metabolism contingent on substrate availability. On the basis of our observations, we are tempted to speculate that human BAT may have the ability to switch metabolism, as we observed that glucose levels did not correlate with BAT-related outcomes (data not shown), whereas omega-3 oxylipins, which were higher in abundance in plasma, negatively correlated with BAT-related outcomes. Further studies are needed to investigate whether this hypothesis is plausible.

Curiously, 12,13-DiHOME is an LA-derived oxylipin secreted by BAT upon cold exposure and exercise (12,13), although in our study, plasma levels of 12,13-DiHOME did not correlate with BAT-related outcomes. In addition, we found that plasma levels of this oxylipin were not related to adiposity and cardiometabolic risk factors of young individuals, whereas it was negatively correlated with lean mass. This finding does not concur with previous evidence showing a negative relationship between the plasma levels of 12,13-DiHOME and BMI and fat mass (12,13), suggesting that more research is needed to understand the use of 12,13-DiHOME as marker of BAT activity and/or metabolic health in humans.

Limitations

As with all cross-sectional studies, the present study does not allow the establishment of causality; however, there are preclinical studies (35,44) supporting the causative role of oxylipins in the development of cardiometabolic diseases. The method for quantifying BAT volume and activity has several limitations, which are described elsewhere (43,45), and the data should therefore be interpreted carefully. The study population included sedentary young adults, which does not allow extrapolation of the findings to older, younger, or unhealthy populations, but it is, as far as we are aware, the only study performed so far. Thus, future studies are needed to evaluate whether these seven oxylipins are markers of cardiometabolic health in aged populations or populations at risk of cardiometabolic diseases.

CONCLUSION

We describe for the first time, to our knowledge, that plasma levels of four omega-6 oxylipins (15-HeTrE, 5-HETE, 14,15-EpETrE, and 8,12-iso-iPF$_{2\alpha}$-VI) positively correlate with adiposity and an adverse cardiometabolic profile, whereas the plasma levels of three omega-3 oxylipins (14,15-DiHETE, 17,18-DiHETE, and 19,20-DiHDPA) negatively correlate with adiposity and a better cardiometabolic profile, in young adults. The panel of seven oxylipins is a better predictor of adiposity than traditional pro- and anti-inflammatory markers. Also, we observed that individuals with obesity have higher plasma levels of omega-6 and lower plasma levels of omega-3 oxylipins than normal-weight peers. We would suggest that this panel of seven oxylipins may be used as an early diagnostic marker for cardiometabolic risk in young adults.○

AUTHOR CONTRIBUTIONS
LJF, FJOP, JVGL, JRR, and BMT conceived and designed the study; LJF, XD, IK, FJOP, WY, and BMT acquired data; LJF and BMT elaborated the statistical analyses section; LJF, TH, EK, ACH, SFV, PCNR, and BMT discussed and interpreted the data; LJF and BMT drafted the manuscript; and all authors critically revised the manuscript. All authors approved the submitted and final version.

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