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High omega-6/omega-3 fatty acid and oxylipin ratio in plasma is linked to an adverse cardiometabolic profile in middle-aged adults

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Abstract

Omega-6 and omega-3 oxylipins may be surrogate markers of systemic inflammation, which is one of the triggers for the development of cardiometabolic disorders. In the current study, we investigated the relationship between plasma levels of omega-6 and omega-3 oxylipins with body composition and cardiometabolic risk factors in middle-aged adults. Seventy-two 72 middle-aged adults (39 women; 53.6±5.1 years old; 26.7±3.8 kg/m^2) were included in this cross-sectional study. Plasma levels of omega-6 and omega-3 fatty acids and oxylipins were determined using targeted lipidomic. Body composition, dietary intake, and cardiometabolic risk factors were assessed with standard methods. The plasma levels of the omega-6 fatty acids and derived oxylipins, the hydroxyeicosatetraenoic acids (HETEs; arachidonic acid (AA)-derived oxylipins) and dihydroxy-eicosatrienoic acids (DiHETrEs; AA-derived oxylipins), were positively associated with glucose metabolism parameters (*i.e.*, insulin levels and homeostatic model assessment of insulin resistance index (HOMA); all $r \geq 0.21$, $P < .05$). In contrast, plasma levels of omega-3 fatty acids and derived oxylipins, specifically hydroxyeicosapentaenoic acids (HEPEs; eicosapentaenoic acid-derived oxylipins), as well as series-3 prostaglandins, were negatively associated with plasma glucose metabolism parameters (*i.e.*, insulin levels, HOMA; all $r \leq 0.20$, $P < .05$). The plasma levels of omega-6 fatty acids and derived oxylipins, HETEs and DiHETrEs were also positively correlated with liver function parameters (*i.e.*, glutamic pyruvic transaminase, gamma-glutamyl transferase (GGT), and fatty liver index; all $r \geq 0.22$ and $P < .05$). In addition, individuals with higher omega-6/omega-3 fatty acid and oxylipin ratio showed higher levels of HOMA, total cholesterol, low-density lipoprotein-cholesterol, triglycerides, and GGT (on average +36%), as well as lower levels of high-density lipoprotein cholesterol (-13%) (all $P < .05$). In conclusion, the omega-6/omega-3 fatty acid and oxylipin ratio, as well as specific omega-6 and omega-3 oxylipins plasma levels, reflect an adverse cardiometabolic profile in terms of higher insulin resistance and impaired liver function in middle-aged adults.

Keywords: PUFAs; Liver function; Insulin resistance; Cardiometabolic profile; Inflammation.

1. Introduction

Omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids that need to be incorporated from the diet [1]. Circulating omega-6 and omega-3 PUFAs can be oxidized enzymatically via cyclooxygenase, lipoxygenase, or cytochrome P450 enzymes or non-enzymatically, leading to the production of oxylipins [2]. Oxylipins are one of the mediators of the effects of PUFAs on human metabolism through binding to G protein-coupled receptors (GPCRs) or peroxisome proliferator-activate receptors (PPARs) [2]. Generally, omega-6 oxylipins have pro-inflammatory properties and can impair immune system functioning, whereas omega-3 oxylipins show opposite effects [2–5].

Preclinical studies have shown that a balanced omega-6/omega-3 oxylipin ratio could exert protective functions against obesity, cardiometabolic and inflammatory diseases, and even cancer [6,7]. In humans, the omega-6/omega-3 PUFA ratio (without characterization of oxylipins) is higher in diabetics *vs.* non-diabetics and is positively correlated with parameters related to insulin resistance (*i.e.*, higher glucose, insulin, homeostatic model assessment of insulin resistance index (HOMA), and glycated hemoglobin [HbA1c]) in type II diabetic patients [8]. Concretely, in humans, omega-6

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oxylipins are involved in the risk or progression of diabetes type II [9], hyperlipidemia [10], and hepatic diseases [11].

Prompt identification of potential individuals at risk of developing cardiometabolic diseases is essential for early treatment [12]. However, the relationship between oxylipins and cardiometabolic risk factors has been exclusively investigated in populations with cardiometabolic complications thus far. Therefore, given the clear link between oxylipins and the incidence of obesity and/or cardiometabolic diseases, it is of clinical interest to investigate the potential role of oxylipins in the development of cardiometabolic diseases in still-healthy individuals. Indeed, omega-6 oxylipins plasma levels have been shown to be positively correlated with adiposity and with an exacerbated cardiometabolic profile in young adults without chronic diseases, whereas omega-3 oxylipins are negatively correlated to adiposity and with a better cardiometabolic profile [13]. Interestingly, plasma levels of omega-6 and omega-3 oxylipins are also better predictors of adiposity than traditional inflammatory markers (e.g., interferon-gamma or tumor necrosis factor-alpha) [13]. However, the relationship between both omega-6 and omega-3 oxylipins and cardiometabolic risk factors in middle-aged adults without cardiometabolic complications has not yet been established.

Thus, we aimed to investigate the relationship between plasma levels of omega-6 and omega-3 oxylipins with body composition and cardiometabolic risk factors in middle-aged adults.

2. Materials and methods

2.1. Research design and participants

The present work is a cross-sectional study within the framework of the FIT-AGEING study (ClinicalTrials.gov. ID: NCT03334357) [14], which involved a total of 72 participants (39 women). The study was approved by the Ethics Committee on Human Research at the University of Granada and "Servicio Andaluz de Salud" (CEI-Granada) [0838-N-2017] and all participants signed informed consent. The study protocol and experimental design were applied in accordance with the last revised ethical guidelines of the Declaration of Helsinki. The inclusion criteria included reporting to be sedentary (*i.e.*, <20 min of moderate-intensity physical activity on 3 d/week over the last 3 months), being free of disease, and having a stable weight over the last 6 months (change <5 kg). The exclusion criteria included being pregnant or lactating women, taking any medication, and/or presenting a major illness that can interfere with or be aggravated by the training program. Participants were requested to be rested, use public transport or a car, refrain from stimulants and/or alcohol on the days of the measurements, and have not performed any moderate exercise in the previous 24 h or vigorous exercise in the previous 48 h.

2.2. Blood collection and determination of plasma oxylipin levels

Blood was collected between 8.00 and 9-00 A.M, after an overnight fast. Blood was drawn from the antecubital vein and was immediately centrifuged to obtain plasma, obtained with Vacutainer Hemogard tubes that contain K_2EDTA as an anticoagulant. Samples were directly aliquoted and stored at -80°C until analysis.

Plasma levels of oxylipins were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [15] using a Shimadzu LC system (Shimadzu Corporation; Kyoto, Japan) connected to a SCIEX QTRAP 6500$^+$ mass spectrometer (SCIEX; Framingham, MA, USA). Of the 78 oxylipins detected (listed in Supplementary Table S1), 47 showed a low analytical variability among quality control (QC) samples with QC_{RSD} ≤15% and 31 showed a moderate variability between 15% <QC_{RSD} ≤30%. Oxylipin results with QC_{RSD} ≤30% were included for further analysis. A detailed description of the whole protocol can be found in the supplementary material. The internal standards used for the LC-MS/MS protocol are listed in Supplementary Table S2.

The LC-MS/MS method allowed for the relative quantitation of oxylipins derived from the omega-6 PUFAs linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), arachidonic acid (AA), and adrenic acid (AdrA), as well as the omega-3 PUFAs α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Individual oxylipins were assigned to fatty acid-derived classifications as reported elsewhere [2].

We computed different groups of fatty acids and oxylipins. These groups were calculated by summing individual data of the fatty acids and/or oxylipins covered by the analytical method. Fatty acids and/or oxylipins included in each group could be found in Supplementary Table S1. The following groups were computed: omega-6 fatty acids and derived oxylipins; LA and derived oxylipins; DGLA and derived oxylipins; AA and derived oxylipins; AdrA and derived oxylipins, hydroxyeicosatetraenoic acids (HETEs); dihydroxy-eicosatrienoic acids (DiHETrEs); series-2 prostaglandins (PGs); isoprostanes (iPFs); omega-3 fatty acids and derived oxylipins; ALA and derived oxylipins; EPA and derived oxylipins; DHA and derived oxylipins, hydroxyeicosapentaenoic acids (HEPEs); series-3 PGs; and, hydroxydocosahexaenoic acids (HDoHEs) (Supplementary Table S1).

2.3. Anthropometric and body composition measurements

Body weight was measured using a model 799 scale and a stadiometer, respectively (both from Seca; Hamburg, Germany), without shoes and with light clothing. Body mass index (BMI) was calculated from weight and height (kg/m^2). Waist circumference (WC) was measured at the minimum perimeter, at the end of a normal expiration, with the arms relaxed at both sides of the body. When the minimum perimeter could not be detected, measurements were taken just above the umbilicus, in a horizontal plane. WC was determined with a plastic tape measure, as the average of two measurements.

Lean mass, fat mass, and visceral adipose tissue (VAT) mass were measured by dual-energy X-ray absorptiometry using a Discovery Wi device (Hologic Inc.; Bedford, MA, USA) equipped with analysis software (APEX version 4.0.2). Lean and fat mass indices were expressed as kg/m^2; fat mass was also expressed as a percentage of body weight.

2.4. Determination of cardiometabolic risk factors

Traditional cardiometabolic risk factors were measured in plasma (*i.e.*, glucose, insulin, total cholesterol, high-density lipoprotein cholesterol [HDL-C], triglycerides, glutamic pyruvic transaminase [GTP], gamma-glutamyl transferase [GGT]). Low-density lipoprotein cholesterol (LDL-C) was calculated from the Friedewald formula. Insulin sensitivity was estimated via the HOMA [16] and the quantitative insulin sensitivity check index (QUICKI) [17]. The fatty liver index was calculated as a validated surrogate marker of non-alcoholic fatty liver disease [18]. Additionally, a sex-specific cardiometabolic risk score was calculated based on the International Diabetes Federation criteria [19]. A detailed description can be found in Supplementary Material.

Lastly, blood pressure was determined in the right arm after a 30-min rest in a supine position, using an Omrom HEM 705 CP automatic monitor (OMROM HealthCare Co.; Kyoto, Japan), following the recommendations of the European Heart Society [20]. Three measurements were taken 1 min apart, and the mean value was calculated.

2.5. Dietary intake

Dietary intake was assessed using three 24-h recalls and a previously validated food frequency questionnaire (FFQ). The three 24-h recalls were performed on three separate days, including two working weekdays and 1 d at the weekend. EvalFINUT software was used to collect information on dietary energy, macronutrient, and lipid intake. The consumed portions for each food group were obtained from the FFQ. Dietary assessment was performed by experienced research dietitians during face-to-face interviews, as described [21,22].

2.6. Statistical analyses

The normal distribution assumption was tested using the Shapiro-Wilk test, visual histograms, and Q-Q plots. Non-normally distributed variables (*i.e.*, cardiometabolic risk parameters and oxylipins) were $\log_{10}$-transformed before further analysis. Since no sex interaction was detected for any parameter (all $P \geq .05$), data from men and women were analyzed together.

The baseline characteristics and outcomes of the study participants were expressed as mean ± standard deviation (unless otherwise stated).

We conducted Pearson partial correlation analyses to examine the relationship between plasma levels of fatty acids and oxylipins and body composition and cardiometabolic risk parameters adjusting for age and fish consumption. 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) [23]. All correlation analyses and plots were designed using the "corrplot" package in R software (V.3.6.0). Figures 2 was built with GraphPad Prism software v.9 (GraphPad Software; San Diego, CA, USA).

Tertiles of the omega-6/omega-3 fatty acids and oxylipins ratio were computed with the *Visual Binning* function in the Statistical Package for the Social Sciences (SPSS) v.25.0 (IBM Corporation; Chicago, IL, USA). Differences in categorical variables between tertiles were analyzed by chi-square tests, whereas differences in continuous variables between groups were analyzed by one-way analyses of variance. Bonferroni *post-hoc* adjustments for multiple comparisons were used to examine differences between low (2.6±0.5; minimum=1.28; maximum=3.11), intermediate (3.6±0.3; minimum=3.2; maximum=4.2), and high (5.3±1.1; minimum=4.3; maximum=9.0) tertiles. The level of significance was set at $P<.05$ after FDR correction.

Table 1
Characteristics of the study participants.

	Total n=72	Men=33	Women=39
Age (years)	53.6±5.1	54.4±5.1	53.0±5.0
BMI (kg/m^2)	26.7±3.8	28.3±3.7	25.3±3.3
Waist circumference (cm)	94.8±11.7	102.6±8.9	88.2±9.7
LMI (kg/m^2)	15.2±2.9	17.5±1.9	13.2±1.8
Fat mass (%)	40.0±9.0	34.6±7.8	44.5±7.4
FMI (kg/m^2)	10.8±3.1	10.0±3.2	11.4±2.9
VAT (g)	786.0±382.6	975.3±383.7	625.8±303.4
Glucose (mg/dL)	93.6±11.3	95.0±13.6	92.4±8.8
Insulin (μUI/mL)	8.1±5.6	8.7±6.7	7.6±4.6
HOMA index	1.9±1.8	2.2±2.4	1.6±1.1
QUICKI	0.4±0.0	0.4±0.0	0.4±0.0
Total cholesterol (mg/dL)	207.2±33.6	203.2±37.1	210.6±30.5
HDL-C (mg/dL)	59.5±12.6	56.2±12.4	62.3±12.2
LDL-C (mg/dL)	126.4±29.5	127.5±32.3	125.5±27.2
Triglycerides (mg/dL)	133.1±63.7	141.5±77.5	126.1±49.1
GPT (IU/L)	23.3±12.6	29.2±13.8	18.3±9.0
GGT (IU/L)	34.4±23.4	41.1±23.7	28.7±21.9
Fatty liver index	49.5±26.3	66.6±20.0	34.8±21.9
SBP (mm Hg)	127.1±15.3	132.5±15.1	122.7±14.1
DBP (mm Hg)	81.2±11.7	83.5±12.1	79.2±11.2
CVD risk score IDF	0.0±0.3	0.0±0.3	0.0±0.3
Energy intake (kcal/d)	2151.4±736.8	2430.5±916.6	1909.0±413.0
Fat intake (g/d)	87.7±25.8	98.4±25.9	78.4±22.2
PUFA intake (g/d)	12.9±5.1	14.2±5.5	11.7±4.5
Fish consumption (servings/week)	0.9±0.4	0.9±0.4	0.9±0.4

Data are presented as mean and standard deviation (SD), otherwise stated.
Abbreviations: BMI, body mass index; CVD risk score IDF, Cardiometabolic risk score of the International Diabetes Federation; DBP, diastolic blood pressure; FMI, fat mass index; GGT, gamma-glutamyl transferase; GPT, glutamic pyruvic transaminase; HDL-C, High-density lipoprotein-cholesterol; HOMA, homeostatic model assessment index; LDL-C, Low-density lipoprotein-cholesterol; LMI, lean mass index; PUFA, polyunsaturated fatty acids; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; VAT, visceral adipose tissue.

3. Results

The characteristics of the participants included in the study are shown in Table 1 (53.6±5.1 years old; 26.7±3.8 kg/m^2), whereas plasma levels of fatty acids and oxylipins are shown in Supplementary Table S3.

3.1. Plasma levels of omega-6 oxylipins positively associate with insulin resistance and omega-3 oxylipins with insulin sensitivity in middle-aged adults

At first step, we observed that the plasma levels of omega-6 fatty acids and derived oxylipins were positively correlated with insulin levels and HOMA (both r≥0.21, P<.05), whereas they were negatively correlated with QUICKI (r=-0.23, all P<.05; Fig. 1). Specifically, the levels of AA and derived oxylipins and AdrA fatty acid were positively correlated with glucose parameters (*i.e.*, insulin and HOMA; all r≥0.25, all P<.05; Fig. 1 and Supplementary Fig. S1). Moreover, among the AA-derived oxylipins, levels of HETEs and DiHETrEs were positively correlated with glucose parameters (*i.e.*, insulin and HOMA; all r≥0.24, all P<.05; Fig. 1 and Supplementary Fig S1).

Surprisingly, also the plasma levels of the omega-3 fatty acid ALA were positively correlated with adiposity and glucose parameters (all r≥0.21, all P<.05; Supplementary Fig. S2). On the other hand, the plasma levels of EPA and derived oxylipins were negatively correlated with insulin and HOMA (both r≤-0.2, P<.05), whereas they were positively correlated with QUICKI (r=0.29, P<.05; Fig. 1 and Supplementary Fig. S2). Concretely, the levels of HEPEs and series-3 PGs were negatively correlated with insulin and HOMA (all r≤-0.20, all P<.05; Fig. 1 and Supplementary Fig. S2).

3.2. Plasma levels of omega-6 hydroxyeicosatetraenoic acids are associated with impaired liver function in middle-aged adults

Additionally, our results revealed that the plasma levels of omega-6 fatty acids and derived oxylipins, and specifically DGLA- and AA fatty acids and derived oxylipins, were positively correlated with GPT, GGT, and fatty liver index (all r≥0.24; all P<.05; Fig. 1). Among the AA-derived oxylipins, the levels of HETEs and DiHETrEs were positively correlated with liver function parameters (*i.e.*, GPT, GGT, and fatty liver index; all r≥0.22; all P<.05; Fig. 1 and Supplementary Fig. S1).

On the other hand, we observed a positive correlation between both the levels of omega-3 and DHA-derived oxylipins and liver function parameters (*i.e.*, GGT; all r≥0.24; all P<.05; Fig. 1). However, it is worth mentioning that the levels of HDoHEs, which could be yielded from DHA autoxidation [24–27], was the main subclass positively correlated with liver function parameters (*i.e.*, GPT, GGT, and fatty liver index; all r≥0.2; all P<.05; Fig. 1 and Supplementary Fig. S2) and also VAT mass (all r≥0.2; all P<.05; Fig. 1 and Supplementary Fig. S2).

All the associations mentioned remained unaltered when dietary energy intake, PUFA intake, and sex were included as confounders (data not shown).

Fig. 1. Pearson correlation analyses between plasma levels of fatty acids and oxylipins groups with body composition and cardiometabolic risk factors in middle-aged adults adjusted by age and fish consumption. Every box represents a significant correlation coefficient after FDR correction (all $P<.05$), whereas empty spaces represent no significant correlations. Blue and red boxes indicate positive and negative correlations, respectively. Number in brackets are the number of oxylipins included in each group. All oxylipins and cardiometabolic risk outcomes were log10-transformed prior to data analysis. The detailed correlation analysis between plasma levels of individual oxylipins with body composition and cardiometabolic risk factors are represented in Supplementary Figures S1 and S2. Abbreviations: AA, arachidonic acid; AdrA, adrenic acid; ALA, α-linolenic acid; BMI, body mass index; CDV risk score IDF, Cardiometabolic risk score of the International Diabetes Federation; DBP, diastolic blood pressure; DGLA, dihomo-γ-linolenic acid; DHA, Docosahexaenoic acid; DiHETrEs, Dihydroxy-eicosatrienoic acids; EPA, eicosapentaenoic acid; FA, fatty acid; FMI, fat mass index; GGT, gamma-glutamyl transferase; GTP, glutamic pyruvic transaminase; HDL-C, High density lipoprotein-cholesterol; HDoHEs, hydroxy-docosahexaenoic acids; HEPEs, hydroxy-eicosapentaenoic acids; HETEs, hydroxyeicosatetraenoic acids; HOMA, homeostatic model assessment index; IPFs, isoprostanes; LA, linoleic acid; LDL-C, Low density lipoprotein-cholesterol; LMI, lean mass index; PGs, prostaglandins; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; VAT, visceral adipose tissue.

3.3. High omega-6/omega-3 fatty acids and oxylipin ratio is linked to an adverse cardiometabolic profile in middle-aged adults

No differences were observed between tertiles of omega-6/omega-3 fatty acids and oxylipin ratio in terms of body composition (*i.e.*, BMI, waist circumference, LMI, fat mass percentage, FMI, or VAT) (Supplementary Table S4). Interestingly, individuals in the highest tertile had significantly higher HOMA (+63.2%), and GGT levels (+65.9%) than participants located in the lowest tertile (Fig. 2 and Supplementary Table S4). By contrast, participants in the highest tertile had lower QUICKI (-7.8%), HDL-C (-12.6%), and fish consumption (-36.4%) compared to individuals in the lowest tertile (Fig. 2 and Supplementary Table S4). Similarly, individuals in the highest tertile had significantly higher total cholesterol (+15.6%), LDL-C (+16.2%), and triglycerides (+45.2%) than participants in the intermediate tertile (Fig. 2). Participants in the highest tertile also displayed lower HDL-C (-13.3%) compared to participants in the intermediate tertile (Fig. 2E**)** Lastly, no differences were observed between tertiles in terms of fatty liver index, blood pressure, and cardiovascular risk score (Supplementary Table S4).

4. Discussion

Here, we demonstrate that the ratio of omega-6/omega-3 fatty acids and oxylipins in plasma is associated with an adverse cardiometabolic profile in middle-aged adults. Likewise, we show that high plasma levels of omega-6 fatty acids and oxylipins are associated with impaired insulin sensitivity and liver function, whereas higher plasma levels of omega-3 fatty acids and oxylipins are associated with improved insulin sensitivity in middle-aged adults. Altogether, our results suggest that omega-6 and omega-3 fatty acids and oxylipins could be positioned as potential candidates to evaluate the cardiometabolic profile in middle-aged adults without any chronic disease.

4.1. Role of omega-6 and omega-3 oxylipins in the regulation of insulin sensitivity

We reveal that elevated plasma levels of omega-6 fatty acids and derived oxylipins, specifically HETEs and DiHETrEs, are associated with impaired insulin sensitivity. Plasma levels of omega-6 oxylipins have been shown to be positively related to pro-inflammatory status and chemokine production [2]. In this context, inflammation plays a causal role in the development of insulin resistance through its contribution to local (*i.e.*, adipose tissue or muscle) and systemic insulin resistance [28]. These oxylipins have various autocrine effects on insulin signaling and metabolism in adipose tissue, skeletal muscle, and liver which contribute to the development of insulin resistance [28]. Specifically, HETEs are AA-derived oxylipins with a marked influence in the generation of leukotrienes and lipoxins [29], which have been linked to obesity [30], cancer, thrombogenesis, cardiovascular diseases, and diabetes

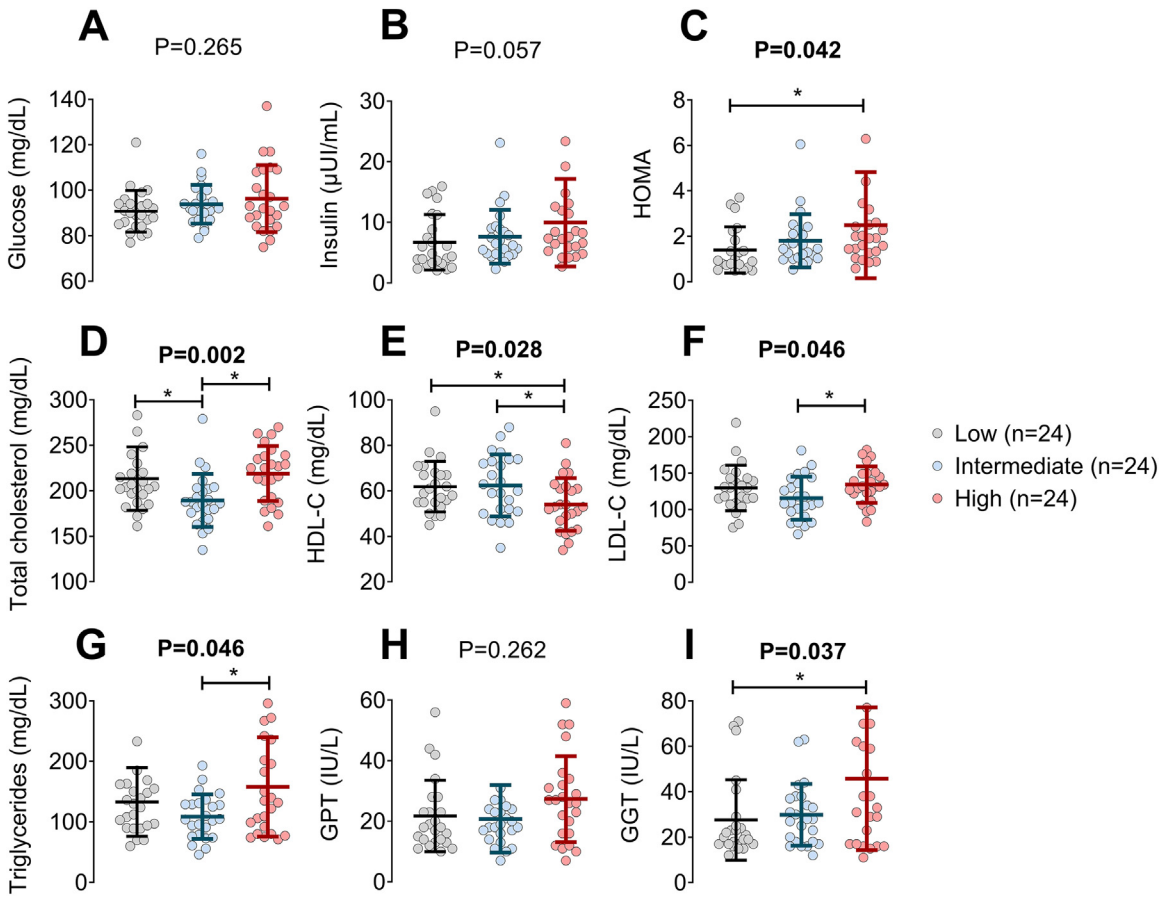

Fig. 2. Comparisons between cardiometabolic risk parameters among participants with low (2.6±0.5; minimum=1.28; maximum=3.11), intermediate (3.6±0.3; minimum=3.2; maximum=4.2), and high (5.3±1.1; minimum=4.3; maximum=9.0), ratio of plasma omega-6/omega-3 fatty acids and oxylipins. *P* value from one-way analysis of variance. *Symbols indicates significant differences between tertiles (*P*<.05) after Bonferroni correction for multiple comparisons. All blood parameters analyses were computed with the log10-transformed variables. Abbreviations: GGT, gamma-glutamyl transferase; GTP, glutamic pyruvic transaminase; HDL-C, High-density lipoprotein-cholesterol; HOMA, homeostatic model assessment; LDL-C, Low-density lipoprotein-cholesterol.

[29,31]. HETEs could impair insulin signaling due to the activation of its receptor GPCR 75, which interferes with the dephosphorylation of the insulin receptor and the translocation of the glucose transporter type 4 (GLUT4) [29,32–34]. It is worth mentioning that the pattern of associations between HETEs and insulin sensitivity parameters could be driven by 20-HETE, which is produced by cytochrome P450, whereas other HETEs are produced by lipoxygenases [2]. On the other hand, DiHETrEs have been demonstrated to impair insulin signaling in preclinical models through organ damage, endoplasmic reticulum stress, and inflammation [35]. High levels of DiHETrEs have been also related to diabetic complications in preclinical studies [35], and type 2 diabetic women displayed higher levels of this group of oxylipins than BMI-matched healthy women [9]. Interestingly, DiHETrEs together with 20-HETE, are also produced by cytochrome P450 enzymes from AA [2], which might suggest that these associations could be explained by an increase in the activity of these enzymes. Therefore, high levels of HETEs and DiHETrEs might contribute to hyperglycemia, insulin resistance, and an increased risk of diabetes mellitus [4,29,31–34].

Contrarily, high plasma levels of omega-3 fatty acids and derived oxylipins, specifically HEPEs and series-3 PGs, are associated with better insulin sensitivity. EPA-derived oxylipins are the intermediate mediators of the EPA metabolic pathway, which leads to the production of series-3 prostacyclins, thromboxanes, and PGs, all presenting anti-inflammatory and cardioprotective functions, as opposed to the omega-6-derived lipid mediators [2,36]. Our results concur with previous evidence suggesting that EPA and its derived products (*i.e.*, HEPEs and series-3 PGs) may improve insulin sensitivity at local level in the liver, adipose tissue, and skeletal muscle [36–38]. Mechanistically, EPA-derived oxylipins can inhibit hepatic lipogenesis through the upregulation of PPARα and the adenine monophosphate-activated protein kinase (AMPK), and the downregulation of the sterol regulatory element-binding transcription factor 1 (SREBP1C) and the carbohydrate-responsive element-binding protein (ChREBP) [36–38]. In adipose tissue, EPA-derived oxylipins can increase fatty acid oxidation and increase the secretion of adiponectin and leptin, as well as decrease adipose tissue inflammation by reducing pro-inflammatory oxylipins through the activation of the GPR120 [36–38]. Lastly, in skeletal muscle, EPA-derived oxylipins prevent the accumulation of fatty acid intermediates via the increments in fatty acid oxidation and the improvement in the inflammatory status and mitochondrial function of skeletal muscle [36–38]. All these mechanisms support the notion that EPA-derived oxylipins could regulate metabolic homeostasis, backing up that the observational findings in the present study may reflect causal relationships.

Contrary, we observed that levels of ALA fatty acid were associated with higher adiposity and worse insulin sensitivity (Supplementary Fig. S2). Adiposity is a major determinant of insulin resistance, and obesity induces insulin resistance which is accompanied by different metabolic dysfunctions [39]. Thus, to avoid the

potential confounder of adiposity in the relationship between ALA and insulin sensitivity, we adjusted the correlation analyses for fat mass percentage, and the associations disappeared (data not shown). It is known that free PUFAs, and concretely ALA, could be stored in adipose tissue and could be released into circulation [40]. Therefore, the lack of association between ALA levels and insulin sensitivity after taking into account adiposity might be due to the strength of the association between adiposity and insulin resistance which influences the results. However, the above-mentioned omega-6 oxylipins, HETEs, DiHETrEs, omega-3 oxylipins, HEPEs, and series-3 PGs, were not correlated with adiposity and therefore these adjustments were not performed.

4.2. Role of oxylipins in the impairment of liver function

We observed that plasma levels of omega-6 fatty acids and derived oxylipins and specifically the HETEs and DiHETrEs are associated with impaired liver function (*i.e.*, higher GPT, GGT, and fatty liver index). In this context, a previous study revealed that patients with acutely decompensated cirrhosis and patients with acute-on-chronic liver failure were characterized by higher levels of pro-inflammatory omega-6 oxylipins and lower levels of omega-3 pro-resolving oxylipins compared to healthy controls [11]. Interestingly, most of the omega-6 oxylipins elevated in acute-on-chronic liver failure and decompensated cirrhosis were AA-derived oxylipins [11]. Although it is important to be aware of the differences between cohorts, our results partially agree with this study. High levels of omega-6 oxylipins increase the overall pro-inflammatory status triggering insulin resistance, fat accumulation, and inflammation in the liver [41]. Concretely HETEs and DiHETrEs lead to vasoconstriction, promote vascular dysfunction, and have pro-inflammatory properties [3,4,42,5], which directly influence hepatic tissue damage and function [41]. Interestingly, these lipid mediators were already related to impaired liver function in middle-aged adults without liver disease, with a marked association with the fatty liver index. The fatty liver index is a recognized predictor of hepatic steatosis in the general population [18], metabolic dysfunction associated with fatty liver disease [43], type diabetes 2 [44], myocardial infarction, stroke, and all-cause mortality [45]. Thus, our results suggest that omega-6 oxylipins could be used as an early marker of liver function in healthy adults which might help to predict future hepatic diseases.

Interestingly, the non-enzymatic free radical oxidation of AA could lead to the synthesis of eight-, nine-, and 11-HETE [46], suggesting a potential role of the oxidative stress in the above-mentioned associations between omega-6 oxylipins and liver function. We also observed that plasma levels of HDoHEs are associated with impaired liver function (*i.e.*, higher GPT, GGT, and fatty liver index). This oxylipin subclass could be yielded via autooxidation [24–27] and has been recognized as a potential marker of oxidative stress [5]. In fact, HDoHEs levels are elevated in patients with hypertension in response to an increase in oxidative stress [5]. Intriguingly, we also observed a positive association between HDoHEs levels and VAT mass, an adipose tissue depot that is clearly associated with increased oxidative stress and a pro-inflammatory status [47]. In this sense, there is a crosstalk between oxidative stress, inflammation, and liver function [48]. In the liver, under oxidative stress conditions, the reactive oxygen species (ROS) could trigger PUFAs autoxidation products [48] which could also worsen oxidative stress and increase the production of pro-inflammatory metabolites with a negative effect on liver function [48]. Therefore, our results suggest that oxylipins produced by autoxidation processes could be related to liver function.

4.3. The omega-6/omega-3 oxylipin ratio as a potential marker of cardiometabolic risk

Our data show that the omega-6/omega-3 oxylipin ratio, a demonstrated surrogate marker of systemic inflammation in previous studies [49], was associated with an adverse cardiometabolic profile (*i.e.*, higher glucose, lipid, and liver function parameters). Previous studies in humans have suggested that an imbalance between circulating omega-6 and omega-3 PUFAs could lead to an increased risk of diabetes type 2 [8,9], hyperlipidemia [10], and hepatic diseases [11]. In addition, pre-clinical studies using the *fat-1* transgenic mouse (which converts omega-6 into omega-3 PUFAs), have demonstrated that an increased omega-3/omega-6 oxylipin ratio underlies the protective phenotype of the *fat-1* mice against obesity and cardiometabolic diseases, such as insulin resistance, liver steatosis, metabolic syndrome, and chronic inflammation [6,7]. Although no studies in humans have thus far reported the relationship between oxylipins ratio and cardiometabolic risk parameters in adults without chronic diseases, our results agree with previous human and preclinical evidence. An unbalanced ratio of oxylipins is driven by higher levels of omega-6 oxylipins and lower levels of omega-3 oxylipins. In this context, high levels of omega-6 oxylipins increase the production of AA-derived eicosanoids (*i.e.,* thromboxanes, prostaglandins, and leukotrienes), which are the last effectors of the pro-inflammatory response [50–52]. Otherwise, low levels of omega-3 oxylipins decrease the generation of anti-inflammatory and pro-resolving lipid mediators (*i.e.*, E- and D-series resolvins, protectins, and maresins), impairing the clearance of pro-inflammatory mediators [50–52]. This imbalance could lead to a systemic pro-inflammatory status that directly adversely affects adipose tissue, liver, pancreas, and immune cells function, and collectively worsens cardiometabolic status as a physiological response [28].

4.4. Limitations

This study is not without limitations. Firstly, the cross-sectional design does not allow the establishment of causality. Secondly, our results should be interpreted with caution due to the limited sample size. Thirdly, no circulating markers of systemic inflammation have been measured in the current study. Due to the low volume of samples, we had to report the area peak ratio as a proxy of the concentration of each metabolite following the Metabolomic Standard Initiative [53]. Lastly, we studied middle-aged sedentary adults only, which does not allow extrapolation of the findings to older, younger, or unhealthy populations. Further studies are thus required to determine whether these results are valid for individuals with different biological characteristics.

5. Conclusion

In summary, our study demonstrated that the omega-6/omega-3 fatty acid and oxylipin ratio is associated with an adverse cardiometabolic profile in middle-aged adults. In addition, high plasma levels of omega-6 fatty acids and oxylipins are linked to impaired insulin sensitivity and liver function, whereas high plasma levels of omega-3 fatty acids and oxylipins are associated with better insulin sensitivity. Plasma levels of omega-6 and omega-3 fatty acids and oxylipins might reflect the cardiovascular status of middle-aged adults, supporting its potential as biomarkers of their cardiometabolic disease risk. Long-term prospective studies are needed to confirm their potential predictive value for cardiovascular diseases and related metabolic diseases.

Author Contributions

LJF, FJOP, BMT, MJC and FJAG conceived and designed the study; LJF, XD, IK, FJOP, WY, and FJAG acquired data; LJF and BMT, elaborated the statically section; LJF and BMT drafted, and all the authors revised the manuscript; all authors read and approved the final manuscript.

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Declaration of competing interests

The authors declare that there are no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2023.109331.

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