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Linoleic acid-derived oxylipins and isoprostanes plasma levels are influenced by 1,25-Dihydroxyvitamin D levels in middle-aged sedentary adults: The FIT-AGEING study

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ABSTRACT

Introduction: Vitamin D – concretely its active form 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) – maintains several physiological processes. Oxylipins are oxidized lipids derived from ω-6 and ω-3 polyunsaturated fatty acids involved in inflammation. Little is known about the association of 1,25$(OH)_2$D with inflammatory parameters in middle-aged populations – who could be at risk of vitamin D deficiency –. The aim of this study was to investigate the relationship between 1,25$(OH)_2$D plasma levels with circulating white blood cells, platelets counts and oxylipins levels.

Materials and methods: A total of 74 (53 % women) middle-aged (40–65 years old) adults were recruited for this cross-sectional study. 1,25$(OH)_2$D plasma levels were measured using an immunochemiluminometric assay. White blood cells and platelets were analyzed by hemocytometry. ω-6 and ω-3 oxylipins plasma levels were measured using liquid chromatography - tandem mass spectrometry. Simple and multiple linear regression models, and Pearson correlation analyses, were performed to study the association of 1,25$(OH)_2$D levels with WBC and platelets counts, and oxylipins, respectively.

Results: 1,25$(OH)_2$D plasma levels were positively related with linoleic acid-derived oxylipins and isoprostanes plasma levels, whereas an inverse relationship with dihomo-γ-linolenic acid/linoleic acid and arachidonic acid/linoleic acid ratios was unveiled. No significant associations were observed for circulating ω-3 oxylipins, white blood cells levels or platelets count.

Conclusions: Linoleic acid-derived oxylipins and isoprostanes plasma levels may be influenced by 1,25$(OH)_2$D plasma levels. Further investigations are needed to elucidate the impact of other vitamin D forms upon circulating oxylipins levels.

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1. Introduction

There is an increased interest in therapeutic strategies to maintain human' health during aging (Reich et al., 2020). The aging process implies important changes in the immune system commonly mediated by an accelerated immunosenescence and pro-inflammatory processes (Rasyid et al., 2021; Akinosun et al., 2021). Vitamin D deficiency represents a growing social problem, affecting >50 % of healthy subjects from any age or ethnicity (Pilz et al., 2018). Although vitamin D has been traditionally related to bone health only, recent evidence has demonstrated its important role on the immune system, i.e., by favoring anti-inflammatory processes (Kheiri et al., 2018).

The role of vitamin D in the regulation of the immune system has been evidenced by the expression of vitamin D receptor (VDR) in immune cells such as lymphocytes, monocytes, and neutrophils (Martens et al., 2020). Previous studies have shown that 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active form of vitamin D – also known as calcitriol – could help to minimize pathogens damage by interacting with an VDR in immune cells (Skrobot et al., 2018), via stimulation of monocytes-induced phagocytosis (Heulens et al., 2016).

Oxylipins are oxidized lipids derived from polyunsaturated fatty acids (PUFAs) and are involved in processes related to inflammation and immunity (Osuna-Prieto et al., 2021). Oxylipins derived from ω-6 fatty acids, such as arachidonic acid (AA) and linoleic acid (LA), tend to increase inflammation, hypertension, and platelet aggregation, whereas oxylipins derived from ω-3 fatty acids present anti-inflammatory, anti-aggregatory, and vasodilatory effects (Gabbs et al., 2015; Caligiuri et al., 2017). Chronic increase in circulating levels of ω-6 oxylipins have shown to be possibly related to degenerative age-related changes at a systemic level (Caligiuri et al., 2017). Moreover, isoprostanes derived from lipid peroxidation present vasoconstrictive and inflammatory properties serv as mediators of inflammatory related disorders (Shoman et al., 2020).

Cardiovascular and inflammatory diseases – which are the number one cause of death globally – are increasing worldwide, and pro-inflammatory oxylipins are augmented in such cardiovascular and inflammatory events (Nayeem, 2018). Additionally, an adequate vitamin D status is involved in anti-inflammatory events via decreasing the expression of pro-inflammatory parameters and may therefore improve quality of life (Latic and Erben, 2020). However, the literature lacks information regarding the possible contribution of $1,25(OH)_2D$ in normal aging, especially in humans, via modulation of pro-inflammatory ω-6 and ω-3 oxylipins. We hypothesize that $1,25(OH)_2D$ plasma levels may play a role in aging by regulating inflammation and immunity processes through the modulation of oxylipins plasma levels.

The present study aimed to examine the association between 1,25 $(OH)_2D$ plasma levels with white blood cells (WBC) levels, platelets count, and oxylipins plasma levels in a healthy cohort of sedentary middle-aged adults.

2. Materials and methods

2.1. Study design and participants

A total of 74 (53 % women) middle-aged (40–65 years old) sedentary adults were recruited using social networks, local media, and posters for this cross-sectional study. The participants were engaged as part of the FIT-AGEING exercise-based randomized controlled trial (clinicaltrial.gov: ID: NCT03334357) (Amaro-Gahete et al., 2018). The inclusion criteria were: (i) age 40–65 years old, (ii) practicing <20 min of physical activity on <3 days per week (i.e., being sedentary), (iii) taking no nutritional supplements, drug or long-term medication (e.g., contraceptive pills and hormonal intrauterine system), (iv) being a non-smoker, (v) having no cardiometabolic illness, (vi) being not pregnant, (vii) having no previous history of liver or kidney disease, (viii) and having no significant weight changes (<3 kg) in the last 12 weeks. The Ethics Committee on Human Research of the Regional Government of Andalucía approved the rationale, design, and methodology of the study [0838-N-2017], and all participants signed a written informed consent in accordance with the Declaration of Helsinki (last revision guidelines, 2013) (World Medical Association, 2013). All blood samples were collected between September and October 2016 and 2017 at the Health Sciences Technology Park (PTS) University Hospital of Granada.

2.2. Body composition parameters

We measured the participant's weight (without shoes and wearing light clothes) and height to the nearest 0.1 kg and 0.1 cm, respectively, using a prevalidated SECA scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany). Body mass index (BMI) was subsequently calculated as weight (kg)/height (m^2). Fat mass (FM) in kg was determined by a dual-energy X-ray absorptiometry scanner (Discovery Wi, Hologic, Inc., Bedford, MA, USA) with analysis software version APEX 4.0.2 to draw an automatic delineation of anatomic regions and following the manufacturer's instructions. The FM index (FMI) was calculated as fat body mass (kg)/height (m^2). FM percentage was calculated by dividing FMI to the total body mass.

2.3. Physical activity and sedentary time assessments

Sedentary and physical activity time were objectively assessed by triaxial accelerometry employing a wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL, USA) for seven consecutive days (24 h/day) (Amaro-Gahete et al., 2018). The sampling frequency was previously set at 100 Hz to store raw accelerations (Migueles et al., 2017). Data were exported and processed using the ActiLife v.6.13.3 software (ActiGraph, Pensacola, FL, USA) and the GGIR package (v.1.5.12; https://cran.r-project.org/web/packages/GGIR) in R software (v.3.1.2; https://www.cran.r-project.org) (Hildebrand et al., 2017; Hildebrand et al., 2014). The participants came to the laboratory and specific information about how to wear the accelerometer was given. They were also reminded to remove it only during water-based activities such as swimming or bathing. Data from participants who did not wear the accelerometers for at least 16 h/day for 4 days (including 1 weekend day) were finally excluded from the analysis.

2.4. Dietary intake measurement

Energy, macronutrient, and micronutrient intakes were assessed using the average of the three 24-h recalls conducted on non-consecutive days (one weekend day included), which has previously demonstrated to determine energy intake within 8–10 % of actual energy intake (Halliday et al., 2014). We used colored photographs of different portion sizes of food to help to estimate the quantity of food consumed (Ruiz López et al., 2010). The interviews were meal-sequence based and involved a detailed assessment and description of the food consumed. Dietary intake from the 24-h recalls was analyzed for energy, macronutrient, and micronutrient contents using the EvalFINUT® software, which is based on the US Department of Agriculture and 'Base de Datos Española de Composición de Alimentos' databases.

2.5. $1,25(OH)_2D$ and blood cells count assessment

10-mL peripheral blood sample was collected using the Vacutainer SST system (Becton Dickinson, Plymouth, UK). All participants were previously requested to abstain from alcohol, and/or caffeine, to eat a standardized dinner (i.e., a standardized proportion of carbohydrates, proteins, and fat), and to avoid any physical activity of moderate (24 h before) and/or vigorous intensity (48 h before). Samples were collected from an antecubital vein of the forearm after a 12-h overnight fast and centrifuged at 4000 rpm for 7 min at 4 °C. Aliquots of plasma obtained with Vacutainer Hemogard tubes, containing the K2 potassium salt of ethylenediaminetetraacetic acid (EDTA) as anticoagulant were stored at

−80 °C until analysis. 1,25(OH)$_2$D plasma levels were measured using a DiaSorin Liaison® immunochemiluminometric analyzer (DiaSorin Ltd., Wokingham, Berkshire, UK) and expressed in pg/mL. WBC (i.e., leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts) and platelet (i.e., mean platelet count and volume) parameters were analyzed using hemocytometry with the Advia 120 Siemens Hematology System (Siemens Healthcare Diagnostics, Erlangen, Germany). These parameters were expressed in μL and fL where appropriate.

2.6. Determination of plasma oxylipins levels

2.6.1. Sample preparation

Oxylipins were extracted using liquid-liquid extraction under ice-cool conditions as previously described (Di Zazzo et al., 2020). Briefly, 150 μL of plasma samples were transferred into 1.5-mL Eppendorf tubes and spiked with 5 μL of a solution of butylated hydroxytoluene (0.4 mg/mL) and 10 μL of a solution containing isotopically labelled internal standards. Then, 150 μL of a buffer solution composed of 0.2 M citric acid and 0.1 M disodium hydrogen phosphate was added prior to the addition of 1000 μL of a 50:50 *v*/v mixture of methyl tert-butyl ether and butanol. Samples were mixed for 5 min using a bullet blender (Next Advance Inc., Troy, NY, USA) prior to centrifugation for 10 min at 16,000 g and 4 °C. The supernatant (900 μL) was collected and evaporated to dryness using a SpeedVac Vacuum Concentrator (Thermo Fisher Scientific, Waltham, MA, USA) prior to reconstitution in 50 μL of a mixture of methanol:acetonitrile (70:30, *v*/v). The reconstituted samples were centrifuged (16,000 *g*, 10 min, 4 °C) prior to collection of 40 μL of the supernatant, which was injected into the chromatograph.

2.6.2. Profiling of oxylipins using liquid chromatography – tandem mass spectrometry

A targeted metabolomics-based approach was used for the relative quantitation of a total of 50 oxylipins derived from the conversion of the ω-6 PUFAs LA, dihomo-γ-linolenic acid (DGLA), AA, and adrenic acid (AdrA); as well as ω-3 PUFAs α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

The oxylipins plasma levels were determined with liquid chromatography – tandem mass spectrometry (LC-MS/MS) (Di Zazzo et al., 2020) using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) connected to a SCIEX QTRAP 6500$^+$ mass spectrometer (SCIEX, Framingham, MA, USA) as previously described (Jurado-Fasoli et al., 2021). Briefly, separation was performed using a BEH C18 column (50 mm × 2.1 mm, 1.7 μm) from Waters Technologies (Mildford, MA, USA) maintained at 40 °C. The mobile phase was composed of 0.1 % acetic acid in water (A), acetonitrile/0.1 % acetic acid in methanol (90:10, *v*/v, (B), and 0.1 % acetic acid in isopropanol (C). The flow rate was set at 0.7 mL/min, whereas the injection volume was 10 μL preceded by the injection of 20 μL of mobile phase. Ionisation of the compounds was performed using electrospray ionisation in negative mode. Selected reaction mode (SRM) was used for MS/MS acquisition. SRM transitions were individually optimised for targeted analytes and respective internal standards using standard solutions.

2.6.3. Data quality and data pre-processing

For each target compound, the ratio between its peak area and the peak area of its respective internal standard was calculated using SCIEX OS-MQ Software and used for further data analysis.

The quality of the data was ensured using regular injection of quality control (QC) samples, consisting of blank plasma samples, within the sequence. QC samples were used to correct for inter-batch variations using the in-house developed mzQuality workflow (available at http://www.mzQuality.nl) (van der Kloet et al., 2009). Relative standard deviations (RSDs) of peak area ratios were calculated for each targeted analyte detected in the QC samples. Metabolites with 15 % ≤ RSDs ≤30 % were marked and should be interpreted with caution. The oxylipins that were detected and relatively quantified by this method are listed in ***Table S1***, whereas the internal standard used are listed in ***Table S2***.

2.7. Statistical analysis

All calculations were performed using the Statistical Package for the Social Sciences v.22.0 (IBM Corporation, Chicago, IL, USA). GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA) was used for the graphical plots. A normal distribution of body composition variables was confirmed using the Shapiro-Wilk test, Q-Q plots, and histograms. None of the WBC and oxylipins plasma levels followed a normal distribution, and were thus Ln and log10-transformed, respectively, for further analyses. The relative intensity (i.e., peak area of the analytes divided by the peak area of its respective internal standard) of LA-, DGLA-, and AA-derived oxylipins were summed from the individual data. The sum of isoprostanes were calculated. Lastly, DGLA/LA and AA/LA ratios were calculated as a proxy of Δ5 and Δ6 desaturase activities, respectively.

Descriptive parameters were presented as mean and standard deviation and compared for sex using an unpaired Student *t*-test as represented in Table 1. Simple linear regression models were performed to study the association of 1,25(OH)$_2$D levels with WBC and platelets counts. Multiple linear regression models were conducted to test these associations after adjusting by sex (Model 1), sex and age (Model 2), as well as sex, age and FMI (Model 3). We conducted Pearson correlation analyses to explore the relationship between 1,25(OH)$_2$D plasma levels and oxylipins plasma levels. Simple and multiple linear regression models were further built as above-mentioned. All previous-mentioned results were additionally adjusted by physical activity levels and nutritional parameters (data not shown). A P value < 0.05 was considered statistically significant.

3. Results

Table 1 shows the descriptive characteristics of the study participants by sex. A total of 31 % of the participants showed normal-weight BMI values, i.e., <25 kg/m^2. Significant differences for sex were observed for BMI and FM (all $P < 0.001$). All blood parameters were similar in both sexes (all $P > 0.05$), except for platelets count which was higher in women than in men ($P = 0.001$).

Table S3 represents the physical activity and dietary intake of the study individuals by sex. Women presented less sedentary time and decreased energy, carbohydrates, calcium, and phosphorus intakes with respect to men (all $P \leq 0.05$).

3.1. 1,25(OH)$_2$D plasma levels are not related to WBC parameters and platelets count

Fig. 1 shows the association of 1,25(OH)$_2$D with WBC parameters (i.e., leukocyte, neutrophil, lymphocyte, monocyte, and eosinophil count) (Fig. 1, Panels A-F) and platelets count (Fig. 1, Panel G). No association was observed between 1,25(OH)$_2$D and WBC or platelets count (all $R^2 \leq 0.168$ and $P > 0.05$) (Fig. 1, Panels A-G), which persisted after controlling for sex (Model 1), sex and age (Model 2), and sex, age and FMI (Model 3). No changes in the present findings have been found after adjusting the analysis by physical activity levels and nutritional parameters (data not shown).

3.2. 1,25(OH)$_2$D plasma levels are related to oxylipins plasma levels

Fig. 2 represents the relationship between 1,25(OH)$_2$D and oxylipins plasma levels. We found that 1,25(OH)$_2$D plasma levels were positively correlated with LA plasma levels; 9,10,13-TriHOME; 9,12,13-TriHOME; and 8,12-isoiPF$_{2\alpha}$-VI (all $r \geq 0.238$, $P \leq 0.044$) (Fig. 2, Panel A). Based on this exploratory analysis, further regression analyses were conducted. We observed a significant direct association between 1,25(OH)$_2$D plasma levels with the sum of LA-derived oxylipins, as well as the sum of

Table 1
Descriptive characteristic of participants.

	N	All		N	Men		N	Women	
Age (years)	74	53.7	(5.1)	35	54.4	(5.3)	39	53	(5.0)
Body composition parameters									
Weight (kg)	74	75.7	(15.0)	35	87.4	(11.0)	39	65.3	(9.3)*
Height (cm)	74	167.8	(9.8)	35	175.8	(6.5)	39	160.7	(6.1)*
Body mass index (kg/m^2)	74	26.7	(3.8)	35	28.3	(3.6)	39	25.3	(3.3)*
Fat mass (%)	74	39.9	(9.1)	35	34.7	(8)	39	44.5	(7.4)*
Fat mass (kg)	74	30.1	(8.5)	35	30.9	(9.8)	39	29.2	(7.1)
Fat mass index (kg/m^2)	74	10.7	(3.1)	35	10	(3.2)	39	11.4	(2.9)
Blood parameters									
1,25(OH)$_2$D (pg/mL)	73	40.3	(14.1)	34	38.3	(13.4)	39	42.0	(14.6)
Leukocyte count (10^3/μL)	73	6.58	(1.60)	34	6.52	(1.8)	39	6.63	(1.43)
Neutrophil count (10^3/μL)	73	3.59	(1.18)	34	3.68	(1.19)	39	3.50	(1.19)
Lymphocyte count (10^3/μL)	73	2.26	(0.67)	34	2.13	(0.49)	39	2.38	(0.78)
Monocyte count (10^3/μL)	73	0.39	(0.11)	34	0.41	(0.12)	39	0.38	(0.10)
Eosinophil count (10^3/μL)	73	0.20	(0.13)	34	0.2	(0.13)	39	0.19	(0.14)
Basophil count (10^3/μL)	73	0.04	(0.02)	34	0.04	(0.02)	39	0.04	(0.03)
Platelet count (10^3/μL)	73	234.4	(46.8)	34	215.7	(44.2)	39	250.7	(43.1)*

Data are presented as means (standard deviation). Unpaired Student T-test was used for comparing mean differences between sex *Significant differences between sexes ($P < 0.05$).

isoprostanes (all $R^2 \leq 0.161$ and all $P \leq 0.05$ (Fig. 2, Panel B and C, respectively). In contrast, 1,25(OH)$_2$D plasma levels showed significant inverse relationships with both DGLA/LA and AA/LA ratios (all $R^2 \leq 0.153$ and all $P \leq 0.006$) (Fig. 2, Panel D and E, respectively). All findings persisted after controlling for sex (Model 1), sex and age (Model 2), and sex, age and FMI (Model 3). No changes in the above-mentioned results were retrieved after adjusting the analysis by physical activity levels and nutritional parameters (data not shown).

4. Discussion

To the best of our knowledge, this is the first study investigating the potential relationship of the active form of vitamin D - 1,25(OH)$_2$D - with the oxylipins plasma profile in a middle-aged healthy cohort. Contrarily to our primary hypothesis, 1,25(OH)$_2$D plasma levels were not related to ω-3 oxylipins, WBC and platelets counts in our study' cohort. However, 1,25(OH)$_2$D plasma levels were directly related to LA-derived oxylipins and isoprostanes plasma levels, whereas an inverse relationship was observed with DGLA/LA and AA/LA ratios.

4.1. 1,25(OH)$_2$D plasma levels are not related to WBC parameters and platelets count

Although 1,25(OH)$_2$D is the vitamin D form responsible of its systemic effects, relevant data of its potential role on WBC count are scarce. It is well known that different WBCs, such as monocytes, or T and B lymphocytes, produce calcitriol (Bivona et al., 2018). Moreover, 1,25 (OH)$_2$D seems to sustain the viability of some leukocytes (e.g., eosinophils), thus exerting its immuno-modulatory role (Calcitriol Reduces Eosinophil Necrosis Which Leads to the Diminished Release of Cytotoxic Granules - FullText - International Archives of Allergy and Immunology, 2016). In our study, no significant associations were found between 1,25 (OH)$_2$D plasma levels and WBC status, which concur with a prior study in which a month of calcitriol supplementation (i.e., 0.75 μg/d) had no effect on WBC count in 88 adults (18–65 years old) (Raoufinejad et al., 2019). Moreover, pre-clinical studies in mice with elevated WBC levels demonstrated that an eight-week calcitriol intervention (i.e., 1.5 μg/kg/d) did not restore WBC to normal values (Kianian et al., 2019).

Previous evidence has shown an inverse relationship between 25-hydroxyvitamin D (25(OH)D) form with platelets count in patients with diabetes and Crohn's disease (Sultan et al., 2019; Xia et al., 2016). This association is likely to be explained by the increased cytokines and pro-inflammatory molecules of these patients typical from these conditions. Nevertheless, data assessing the association between 1,25(OH)$_2$D and platelets count in healthy humans have not been addressed. We observed no association between 1,25(OH)$_2$D levels and platelets count in our study' cohort. However, recent investigations have showed elevated platelets counts in patients with acute coronary syndromes who presented reduced plasma 1,25(OH)$_2$D levels (Saghir Afifeh et al., 2021). This relationship might be justified by the role of calcitriol on modulating the coagulation cascade, concretely by increasing thrombomodulin expression. Agwu and Holub (1983) reported that 1,25 (OH)$_2$D exerts its function as a proaggregating agent via increasing calcium (Ca) mobilization and availability for platelets activation in both mice/rats and humans. Along with this, platelets formation and aggregation depend on proper Ca^{2+} fluxes, which are facilitated by the 1,25(OH)$_2$D binding to its VDRs (Sultan et al., 2019). Given that the VDRs seem to be highly expressed in platelets to ensure their Ca^{2+}-dependent functions (Silvagno et al., 2010), the non-genomic influence of vitamin D on platelets function modulating their activity seems obvious with recent studies supporting that vitamin D – especially calcitriol – could decrease platelets aggregation, it seems that it is effective only when administering supraphysiological doses of calcitriol (10 nM) showing a non-genomic effect of vitamin D on platelets (Sultan et al., 2019).

4.2. 1,25(OH)$_2$D plasma levels are related to oxylipins plasma levels

Our results showed a direct association between 1,25(OH)$_2$D levels and LA-derived pro-inflammatory oxylipins. These results concur with those reported by one study developed in rats (Tsutsumi et al., 1985). The authors found that when vitamin D levels were depleted, LA plasma concentrations were subsequently decreased. Interestingly, once vitamin D was repleted, LA levels were restored (Tsutsumi et al., 1985). Moreover, another study observed that the presence of PUFAs, such as LA, decreased the affinity of vitamin D-binding protein (DBP) to 1,25 (OH)$_2$D, thereby increasing the 1,25(OH)$_2$D circulating levels (Bouillon et al., 1992). We also noted that 1,25(OH)$_2$D plasma levels were directly related to isoprostanes plasma levels. Opposite to our findings, the increments in 1,25(OH)$_2$D levels in placental trophoblasts cells decreased the circulating isoprostanes production by blocking their intermediary pathways (Sun et al., 2014). Similarly, mice supplemented with 1,25 (OH)$_2$D reduced their circulating levels of isoprostanes after intervention (Zhu et al., 2017). However, further studies are needed to

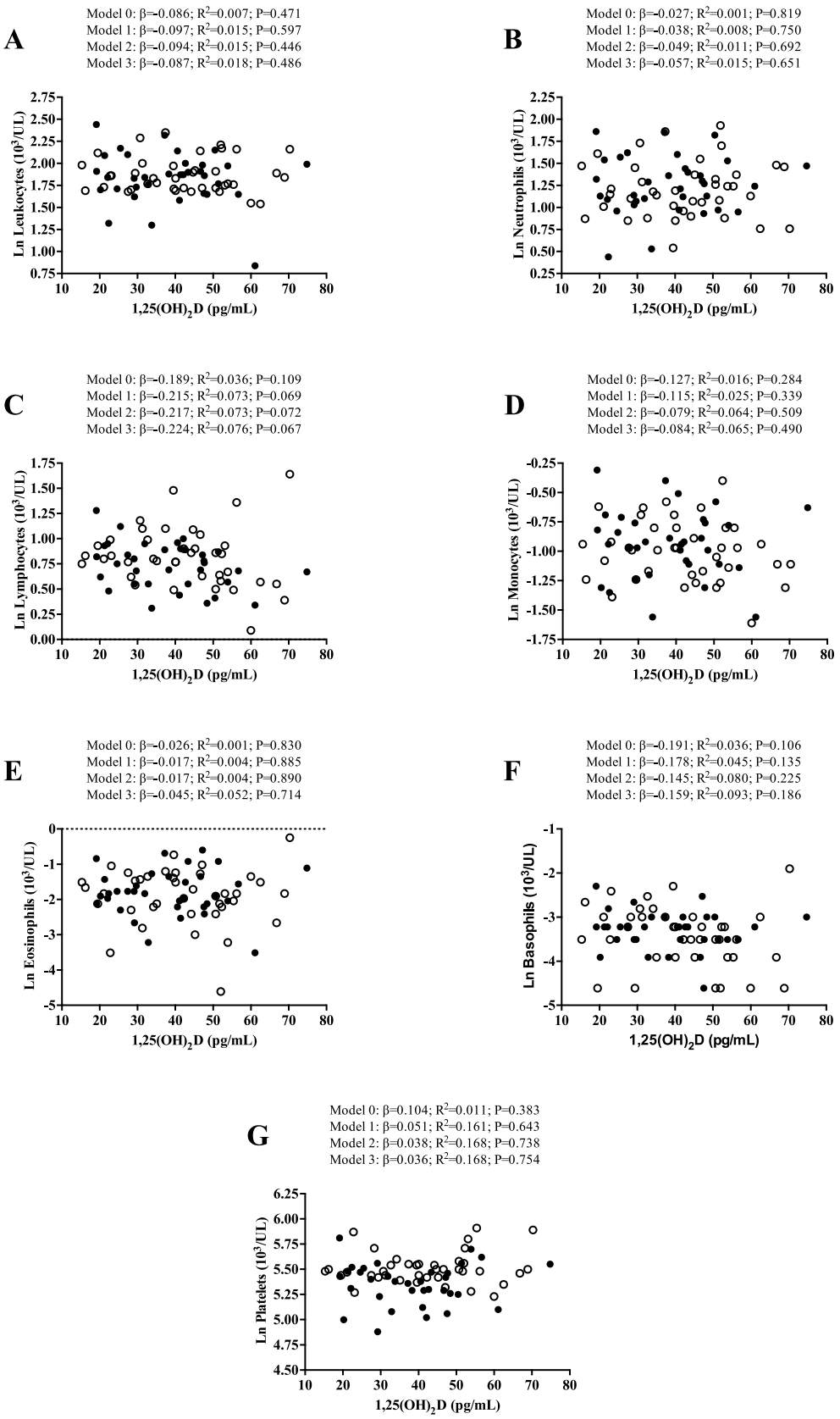

Fig. 1. Association of $1,25(OH)_2D$ levels with leukocytes count (Panel A), neutrophils count (Panel B), lymphocytes count (Panel C), monocytes count (Panel D), eosinophils count (Panel E), basophils count (Panel F) and platelets count (Panel G) in middle-aged healthy sedentary adults. Black dots represent individual observations for man while white dots represent individual observations for women (white dots). β (standardized regression coefficient) and P from multiple linear regression analyses. *P* value < 0.05 was considered statistically significant. Model 0: unadjusted; Model 1; adjusted by sex; Model 2: adjusted by sex and age; Model 3: adjusted by sex, age, and fat mass index.

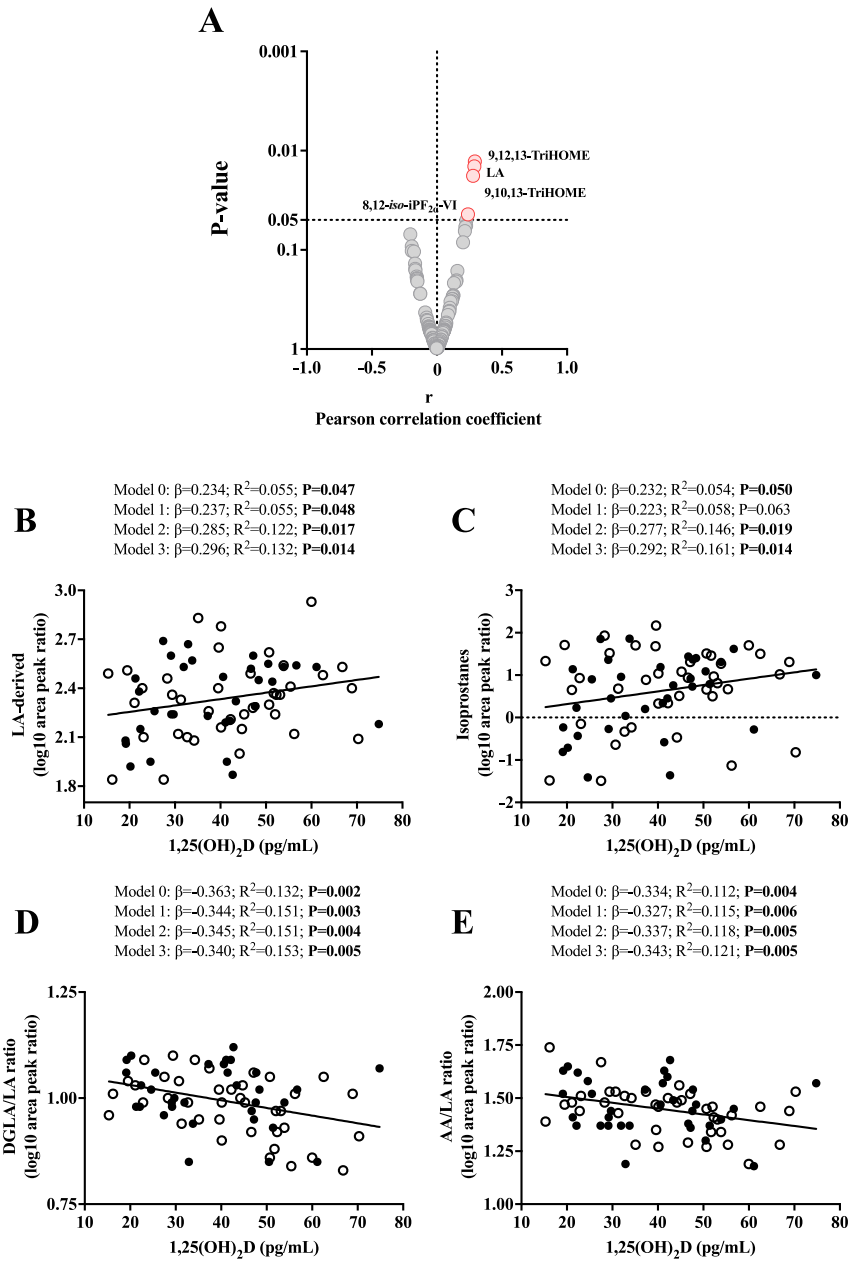

Fig. 2. Relationship between $1,25(OH)_2D$ plasma levels and oxylipins plasma levels. Panel A: Volcano plot showing correlations between $1,25(OH)_2D$ levels and individual oxylipins plasma levels. The X-axis represents the Pearson correlation coefficient, whereas the Y-axis represents the *P* values of the correlations. Only significant correlations ($P < 0.05$) were annotated with the name of the oxylipin. Red dots represent positive significant correlations whereas grey dots represent non-significant correlations. Panels B-E: Linear regression models of 1,25 $(OH)_2D$ levels with the sum of LA-derived oxylipins (Panel B), the sum of isoprostanes (Panel C), the DGLA/LA ratio (Panel D) and the AA/LA ratio (Panel E). Black dots represent individual observations for man while white dots represent individual observations for women (white dots). β (standardized regression coefficient) and P from multiple linear regression analyses. P value < 0.05 was considered statistically significant. Model 0: unadjusted; Model 1; adjusted by sex; Model 2: adjusted by sex and age; Model 3: adjusted by sex, age, and fat mass index. Abbreviations: LA = linoleic acid; DGLA = dihomo-γ-linolenic acid; AA = arachidonic acid.

investigate these associations in humans, especially for the direct relationships observed between 9,12,13-TriHOME, 9,10,13-TriHOME, 8,12-isoIP$_{2\alpha}$-VI, and $1,25(OH)_2D$, which lack of scientific literature evaluating such associations. In this regard, TriHOMEs, especially 9,12,13-TriHOME and 9,10,13-TriHOME, although are the most well-described TriHOME regioisomers, little is known about their physiological relevance in inflammatory conditions (Fuchs et al., 2018). Moreover, 8,12-isoIP$_{2\alpha}$-VI is a highly oxidizable compound involved in the pathogenesis of inflammatory processes (Korecka et al., 2010). As $1,25(OH)_2D$ has been reported to be elevated in pathological processes and to be positively correlated to both inflammatory processes and parameters (Mangin et al., 2014), the findings of our study could be partially elucidated by the previous-mentioned statement.

In our study, $1,25(OH)_2D$ plasma levels showed an inverse association with DGLA/LA and AA/LA which could be considered proxies of Δ5 and Δ6 desaturase activities, respectively. In this regard, Nandy et al. concluded that diet-induced vitamin D deficiency was related to an increased estimated Δ5-desaturase activity index in plasma but did not influence Δ6-desaturase activity in rats (Nandi et al., 2019). Therefore, increased levels of vitamin D could limit the production of DGLA and AA-derived oxylipins, prioritizing their maintenance as LA-derived oxylipins. Indeed, compared to oxylipins derived from DGLA and AA, those derived from LA are less pro-inflammatory and impair the immune system to a lesser extent (Jurado-Fasoli et al., 2021).

4.3. Limitations and strengths

The present study suffers from some limitations including: (i) its cross-sectional design, which means that no causal relationships can be established; (ii) the study cohort was limited to sedentary healthy middle-aged adults (45–65 years old) and hence these results may not be generalizable to younger, older, and/or physically active individuals; (iii) the menopausal situation of the women enrolled in the study which could influence the inflammatory status was not considered; (iv) no

information of C-reactive protein and other proinflammatory parameters was available for logistical reasons; (v) no data about ω-6 poly unsaturated fatty acid consumption was obtained; (vi) we did not measure soy oil consumption, which is rich in DGLA-dihomo-gamma linolenic acid, and therefore, future studies are required to determine whether DGLA-dihomo-gamma linolenic acid concentrations are related to soy oil consumption; (vii) the fact that (25(OH)D) levels were not determined, which would have allowed to better understand the role of different forms of this vitamin in relation with the rest of blood parameters analyzed. In this regard, 25(OH)D is a marker of vitamin D status, and $1,25(OH)_2D$ reflects the activity of vitamin D, presenting opposite functions. Thus, these results should be interpreted with caution, being necessary further studies addressing the analysis of both vitamin D forms to reinforce our findings (Iglar and Hogan, 2015). As strengths, this is the first trial which assesses the relationship of circulating vitamin D and ω-6 and ω-3 oxylipins in humans. We measured several oxylipins by LC-MS/MS, allowing to have a deeper insight into the inflammatory status of the individuals.

5. Conclusions

For the first time, we show that linoleic acid-derived oxylipins and isoprostanes plasma levels may be influenced by $1,25(OH)_2D$ plasma levels. Furthermore, $1,25(OH)_2D$ plasma levels showed no relationship with ω-3 oxylipins, WBC and platelets count. Further investigations are needed to elucidate the influence of other vitamin D forms upon both novel and traditional inflammatory markers.

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Declaration of competing interest

None.

Data availability

Data will be shared upon reasonable request to corresponding authors. Héctor Vázquez-Lorente (hectorvazquez@ugr.es) & Francisco J. Amaro-Gahete (amarof@ugr.es). Department of Physiology, University of Granada, Granada, Spain.

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CRediT authorship contribution statement

Héctor Vázquez-Lorente: data curation, formal analysis, validation, visualization, roles/writing - original draft and writing - review & editing; Lucas Jurado-Fasoli: data curation, formal analysis, software and writing - review & editing; Isabelle Kohler: investigation and review & editing; Xinyu Di: investigation and review & editing; Wei Yang: investigation and review & editing; Francisco J. Osuna-Prieto: methodology, supervision and review & editing; Sardor Asadov: investigation and review & editing; José F. Frias-Rodríguez: methodology, supervision and review & editing; Manuel J. Castillo-Garzón: conceptualization, funding acquisition, project administration and resource and review & editing; Francisco J. Amaro-Gahete: conceptualization, funding acquisition, project administration, resources, validation and writing - review & editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2022.111954.

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