Lipid signaling and inflammation: metabolomics for better diagnosis and treatment strategy

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Chapter VI

Effect of different exercise training modalities on fasting levels of oxylipins and endocannabinoids in middle-aged sedentary adults: a randomized controlled trial

Based on:

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Abstract

This study aimed to investigate the effects of different exercise training programs on fasting plasma levels of oxylipins, endocannabinoids (eCBs), and eCBs-like molecules in middle-aged sedentary adults. A 12-week randomised controlled trial was conducted using a parallel-group design. 65 middle-aged adults (40-65 years old) were randomly assigned to: (1) no exercise (control group), (2) concurrent training based on international physical activity recommendations (PAR group), (3) high-intensity interval training (HIIT group), and (4) high-intensity interval training together with whole-body electromyostimulation (HIIT+EMS group). Plasma levels of oxylipins, eCBs, and eCBs-like molecules were determined in plasma samples before and after the intervention using targeted lipidomics. Body composition was assessed through dual-energy X-ray absorptiometry, and dietary intake through a food frequency questionnaire and three non-consecutive 24 h recalls. The PAR, HIIT, and HIIT+EMS groups, showed decreased plasma levels of omega-6 and omega-3 derived oxylipins after 12 weeks (all $\Delta \leq -0.12$; all $P < 0.05$). However, after Bonferroni post-hoc corrections, the difference was not statistically significant compared with the control group (all $P > 0.05$). Similarly, HIIT and HIIT + EMS showed decreased plasma levels of eCBs and eCBs-like molecules (all $\Delta \leq -0.05$; all $P < 0.05$) while the difference was not significant different from the control group after Bonferroni post-hoc corrections (all $P > 0.05$). However, after post-hoc corrections, plasma levels of anandamide (AEA) and oleoyl-ethanolamide (OEA) were increased in the PAR group compared with the control group (AEA: $\Delta = 0.05$ vs. -0.09; OEA: $\Delta = -0.12$ vs. 0.013, all $P \leq 0.049$). In conclusion, this study reports that a 12-week exercise training intervention, independently of the modality applied, does not modify fasting plasma levels of omega-6 and omega-3 oxylipins, eCBs and eCBs-like molecules in middle-aged sedentary adults.

Introduction

Oxylipins and endocannabinoids (eCBs) are a broad group of bioactive lipids derived from polyunsaturated fatty acids (PUFAs) with autocrine, paracrine, and endocrine functions [1,2]. Oxylipins are the main mediators of the PUFAs physiological effects, regulating the vascular tone, immune function, inflammation processes, and adipose tissue functions [2-4]. Generally, omega-6 oxylipins are pro-inflammatory and show cardiodepressive effects, whereas omega-3 oxylipins lead to the opposite effects [2,5-7]. eCBs and eCBs-like molecules are lipid mediators, which play an important role in brain health, adipose tissue functions, insulin signalling, inflammatory response, and satiation/food intake regulation [1,2,8].

Besides these physiological functions, these lipid mediators have been also proposed as potential regulators of the immune and inflammatory orchestra in response to exercise [9,10]. Indeed, both acute endurance and resistance exercises induced changes in plasma levels of a high number of oxylipins, dependent on the type of exercise and its intensity [10]. The plasma levels of the eCB anandamide (AEA) also increased in response to acute endurance exercise with potential effects in analgesia, leading to the so-called *runner's high* [9]. Both acute endurance and resistance exercise increase gene expression of the cannabinoids receptors (i.e., CB1 and CB2) and the synthesis and degradation of enzymes involved the eCB system in skeletal muscle in humans, all of these likely contributing to the regulation of the plasma levels of eCBs and eCBs-like molecules [9].

While acute exercise, especially endurance exercise, is known to increase plasma levels of omega-6 and omega-3 oxylipins, eCBs and eCBs-like molecules [9-16], the effects of chronic exercise training on their fasting levels has been poorly explored. So far, two studies only have investigated the effects of chronic endurance exercise on isoprostanes and prostaglandins in humans, but did not include the broader coverage of the metabolites of the omega-6 and omega-3 oxylipins pathways [17,18]. Conversely, chronic aerobic, endurance and concurrent exercise training did not modify the gene expression of the eCB receptors in human's skeletal muscle [9]. Given the clear benefits of exercise training on the inflammatory status and the immune system [19], it seems plausible that these health benefits may be partially explained by the influence of exercise training on fasting levels of oxylipins, eCBs, and eCBs-like molecules. The aim of the present study was to investigate

the effects of different modalities of exercise training during 12 weeks, [i.e., concurrent training based on international physical activity recommendations (PAR group), high-intensity interval training (HIIT group), and high-intensity interval training together with whole-body electromyostimulation (HIIT+EMS group)], on fasting plasma levels of oxylipins, eCBs, and eCBs-like molecules in middle-aged sedentary adults.

Methods

Study participants

Eighty-nine nine middle-aged (40-65 years old) sedentary adults participated in the FIT-AGEING study (clinicaltrial.gov: ID: NCT03334357) [20]. Participants were recruited through social networks, local media, and posters. The inclusion criteria included (i) the reporting of being sedentary (i.e., <20 min of moderate-intensity physical activity on <3 days/week over the last 3 months), (ii) no chronic or acute disease, and (iii) having a stable weight over the last 6 months (change <5kg). The exclusion criteria included (i) pregnancy or lactation, (ii) the use of chronic medication and/or (iii) presenting a major illness that can interfere with or be aggravated by an exercise training program. 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 an informed consent prior to inclusion in the study. The study protocol and experimental design were applied in accordance with the last revised ethical guidelines of the Declaration of Helsinki (2013).

Study design

A 12-week randomized controlled trial was conducted with a parallel group design. The study was conducted during two consecutive years from September to December of 2016 and 2017 in two different phases. After baseline examination, the participants were randomly assigned into four different groups using a computer-generated simple randomization [21]: (i) control group (no exercise), (ii) concurrent training based on international physical activity recommendations (PAR group), (iii) high-intensity interval

training group (HIIT group) and (iv) high-intensity interval training adding whole-body electromyostimulation group (HIIT+EMS group). The participant's randomization assignment was blinded to the assessment staff. All participants were requested to maintain their dietary habits. Individuals assigned to the control group were also requested not to change their physical activity habits or to engage in any kind of physical training program, whereas individuals in the exercise groups were instructed to avoid additional exercise to their intervention programs.

Exercise training modalities

A detailed description of each training modality can be found elsewhere [20]. All training sessions were performed in groups of 2–6 participants and a gradual progression was also scheduled to ensure a good adherence to each intervention group. Additionally, training sessions started in all groups with a dynamic standardized warm-up and ended with an active global stretching cooling down protocol.

The PAR group performed 3 concurrent training sessions per week. The endurance training involved a total of 150 min/week at 60-65% of the heart rate reserve, and ∼60 min/week at 40–50% of one repetition maximum were completed for the resistance training.

The HIIT group performed 2 training sessions per week involving 2 different and alternative high-intensity interval training protocols based on long-intervals (LI) and short-intervals (SI) [22]. A volume of 40–65 min/week was established and the intensity was set at > 95% of the maximum oxygen uptake (VO_{2max}) in LI, and 6–9 of the ratings of perceived exertion scale [23] in SI, respectively. LI sessions were performed using a treadmill with a personalized slope, whereas 8 different weight-bearing exercises in circuit form were used for the SI sessions.

The training program performed by the HIIT+EMS group had similar characteristics to the HIIT group, with the addition of whole-body electromyostimulation using a dedicated wireless device (Wiemspro®, Malaga, Spain). The electric pulse was bipolar, symmetrical, and rectangular with a frequency of 15–20 Hz in LI and 35–75 Hz in SI, respectively; an intensity of 100 milliamps in LI and 80 milliamps in SI; respectively; an impulse breadth

of 200–400μs in both LI and SI; and a duty cycle of 99% in LI and 50–63% in SI, respectively.

Blood sample collection

Blood samples were collected in the morning (8:30 AM-10 AM) after overnight-fast (i.e., ≥ 12 h) in Vacutainer® Hemogard™ tubes, containing a K2 potassium salt of ethylenediaminetetraacetic acid as anticoagulant. All samples were centrifuged at 4,000 rpm for 7 min at 4°C. The aliquots of plasma were stored at −80°C until analysis.

All participants were asked to abstain from drugs, alcohol, and/or caffeine, to eat a standardised dinner, and to avoid any physical activity of moderate intensity (24 h before) and/or vigorous intensity (48 h before). After the intervention, blood samples were collected 72- 96 h the last bout of exercise.

Determination of plasma oxylipins, endocannabinoids and endocannabinoids-like metabolites

A targeted metabolomics-based approach was used for the determination of the relative quantitation of oxylipins derived from the conversion of the omega-6 PUFAs linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), arachidonic acid (AA), and adrenic acid (AdrA); the omega-3 PUFAs α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA); the eCBs AEA and 2-arachidonoylglycerol (2-AG); as well as the eCBs-like molecules such as N-acetylethanolamines. The oxylipins, eCBs and eCBs-like molecules included in the method are listed in **Table S1.** The area peak ratio of all omega-6-, LA-, DGLA-, AA-, HETEs-, omega-3-, ALA-, EPA-, HEPEs-, DHA-, and HDoHEs-derived oxylipins covered by the analytical method were summed from the individual data.

The plasma levels of oxylipins, eCBs and eCBs-like molecules were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) [24] with a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan; and a BEH C18 column, Waters Technologies, Mildford, MA, USA) connected to a SCIEX QTRAP 6500^{+} mass spectrometer (AB Sciex, Framingham, MA, USA). The internal standards used are in **Table**

S2. The quality of the data was ensured using regular injection of quality control (QC) samples.

-Sample Preparation

Oxylipins, eCBs, and eCBs-like molecules were extracted using liquid–liquid extraction as previously described [24]. Briefly, 150 µl of plasma samples were transferred into 1.5ml 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 0.2M citric acid and 0.1M disodium hydrogen phosphate was added prior to the addition of 1,000 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.) prior to centrifugation for 10 min at 16,000g and 4°C. The supernatant (900 µl) was collected and evaporated to dryness using a SpeedVac Vacuum Concentrator (ThermoFisher Scientific) prior to reconstitution in 50 µl of a mixture of methanol:acetonitrile (70:30, v/v). The reconstituted samples were centrifuged (16,000g, 10 min, 4°C) prior to collection of 40 µl of the supernatant, which was injected into the LC–MS/MS instrument.

-Liquid Chromatography—Tandem Mass Spectrometry

The analysis was performed using a validated method [24]. Briefly, extracted samples were analyzed using a Shimadzu LC system (Shimadzu Corporation) hyphenated to a SCIEX QTRAP 6500+ mass spectrometer (SCIEX). The separation was performed using a BEHC18 column (50mm× 2.1mm, 1.7 µm) from Waters Technologies maintained at 40°C. The mobile phase was composed of 0.1% acetic acid in water (A), acetonitrile/methanol (90:10, v/v) with 0.1% acetic acid (B), and 0.1% acetic acid in isopropanol (C). Ionization of the compounds was performed using electrospray ionization in negative mode and positive mode with polarity switching. Selected Reaction Mode was used for MS/MS acquisition. Selected Reaction Monitoring transitions were individually optimized for targeted analytes (see **Supplementary Table S1**) and respective internal standards using standard solutions (see **Supplementary Table S2**).

-Data Quality and Data Preprocessing

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 monitored using regular injection of quality control (QC) samples, consisting of blank plasma samples, within the sequence. QC samples were used to correct for interbatch variations using the in-house developedmzQuality workflow (available at http://www.mzQuality.nl) [25]. Relative standard deviations (RSDs) of peak area ratios were calculated for each targeted analyte detected in the QC samples.

In total, 89 oxylipins, eCBs, and eCBs-like molecules were detected. Among them, 53 metabolites showed an acceptable quality (i.e., low analytical variability with RSD of the quality control [QCRSD] $\leqslant$ 15%,), and 36 metabolites showed a moderate variability, that is, 15% < QCRSD $\leqslant$ 30%.

Anthropometric and body composition measurements

Body weight and height were measured without shoes and with light clothing using a model 799 scale and a stadiometer, respectively (both from Seca, Hamburg, Germany). Body mass index (BMI) was calculated from the weight and height (kg/m^2). Waist circumference (WC) was measured at the minimum perimeter, after a normal expiration, and 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. WC was measured twice with a plastic tape measure; the two measures were averaged for further analyses.

Lean, fat and visceral adipose tissue masses were measured by dual-energy X-ray absorptiometry using a Discovery Wi device (Hologic Inc., Bedford, MA, USA) equipped with the analysis software APEX (version 4.0.2).

Physical fitness assessment

Cardiorespiratory fitness was determined through a maximum effort test on a treadmill (Pulsar treadmill, H/P/Cosmos Sports & Medical GmbH, Nussdorf-Traunstein, Germany) following the modified Balke protocol [26], whereas muscular strength was assessed through the handgrip strength test. An extensive description of both tests can be found elsewhere [27].

Dietary intake

Dietary intake was assessed using a previously validated food frequency questionnaire (FFQ) and three 24 h-recalls undertaken on three separate days, as previously described [28]. The portion consumption of fish and nuts were estimated from the FFQ considering the standard portion of each food item [29]. The PUFA intake recorded in the 24 h-recalls was determined using EvalFINUT® software.

Statistical analyses

The statistical analyses were performed using the Statistical Package for the Social Sciences v.26.0 (IBM Corporation, Chicago, IL, USA) and figures were built with GraphPad Prism software v.9 (GraphPad Software, San Diego, CA, USA). The significance threshold was set at $P < 0.05$.

Descriptive data are expressed as mean ± standard deviation, unless otherwise stated. First, data normality was assessed using the Shapiro-Wilk test, visual histograms and Q-Q plots. None of the oxylipins, eCBs and eCBs-like molecules plasma levels followed a normal distribution, and were thus log10-transformed for further analyses.

During exploratory analyses, paired T-tests were performed to assess the effect of the 12-week exercise intervention on plasma levels of oxylipins, eCBs, and eCBs-like molecules in each group separately. Then, a delta (Δ; post - baseline log10 values) was calculated for every outcome. Changes in plasma levels of oxylipins eCBs and eCBs-like molecules in each group (i.e., control, PAR, HIIT and HIIT+EMS) were assessed with metabolic pathway analyses using Cytoscape software (version 3.7.0; version3.7.0, Institute for Systems Biology) [30].

In order to assess the effects of the different exercise modalities on plasma levels of oxylipins, eCBs, and eCBs-like molecules, analyses of covariance (ANCOVA) were conducted, including the corresponding baseline value as covariate. Bonferroni post-hoc adjustments for multiple comparisons were used to examine differences between groups. Additional ANCOVAs were conducted to assess the effects of the different exercise modalities on plasma levels of oxylipins, eCBs, and eCBs-like molecules including baseline values of these molecules, as well as PUFA, fish and nut intake as covariates. Further, to

account for multiple testing across oxylipins, eCBs, and and eCBs-like molecules changes, we next used the two-stage step-up method of Benjamini-Hochberg false discovery rate (FDR) method, using a FDR of 0.25 [31].

Results

Among the 89 participants allocated to the four groups, a total of 18 participants dropped out of the study during the intervention (**Figure S1**), including 6 participants excluded from the main analyses due to problems in blood sample collection. The characteristics of the 65 participants (52% women) included in the analyses are shown in **Table 1**.

Table 1. Baseline characteristics of the study participants.

	Control (n=14)	PAR (n=17)	HIIT (n=17)	HIIT + EMS (n=17)
Demographics				
Age (years old)	51.8 ± 4.2	54.9 ± 4.5	53.5 ± 5.6	53.7 ± 5.4
Male (n; %)	6 , 42.9	8 , 47.1	8 , 47.1	9 , 52.9
Female (n; %)	8 , 57.1	9 , 52.9	9 , 52.9	8 , 47.1
Body composition				
BMI (kg/m2)	26.4 ± 3.8	25.4 ± 2.9	26.4 ± 3.2	29 ± 4.5
Waist circumference (cm)	92.5 ± 10.8	90.4 ± 11	97.5 ± 11.2	100.2 ± 13.5
Lean mass (kg)	44 ± 12	43.6 ± 10.8	43.3 ± 13.1	45.3 ± 10.6
Fat mass (kg)	26.9 ± 6.1	26.8 ± 6.3	31.8 ± 8.3	33.8 ± 10.4
Fat mass (%)	37.3 ± 8.4	37.4 ± 8.8	41.6 ± 8.1	41.2 ± 9
VAT mass (g)	701 ± 273	661 ± 263	837 ± 455	967 ± 485
Physical Fitness				
Handgrip strength (kg)	70.5 ± 25.3	72 ± 25	70.7 ± 27.6	73.4 ± 20
VO2peak (ml/min)	2204 ± 634	2320 ± 650	2394 ± 667	2429 ± 662
VO2peak (ml/kg/min)	30 ± 5	32 ± 6	31 ± 6	30 ± 5
Time to exhaustion (s)	837 ± 246	797 ± 214	804 ± 237	931 ± 202

Data are presented as mean ± standard deviation unless otherwise stated. *Abbreviations*: BMI: body mass index; HIIT, high-intensity interval training group; HIIT+EMS, high-intensity interval training + whole-body electromyostimulation group; PAR, physical activity recommendations group; VAT: visceral adipose tissue; VO_2: volume of oxygen.

Effect of different exercise training modalities on plasma levels of oxylipins

Overall, we observed that the 12-week exercise intervention decreased plasma levels of oxylipins in the PAR, HIIT, and HIIT+EMS groups (**Figures 1B, C, and D**). Specifically, we found that PAR group decreased plasma levels of omega-3- (Δ = -0.18; P = 0.008), EPA- (Δ = -0.25; P = 0.013), HEPEs (Δ = -1.28; P = 0.010), DHA- (Δ = -0.16; P = 0.014), and HDoHEs- (Δ = -1.74; P = 0.019) derived oxylipins. We also observed that HIIT group decreased plasma levels of omega-6- (Δ = -0.12; P = 0.043), LA- (Δ = -0.16; P = 0.027), DGLA- (Δ = -0.12; P = 0.035), HETEs- (Δ = -0.84; P = 0.039), omega-3- (Δ = -0.23; P = 0.006), EPA- (Δ = -0.24; P = 0.014), HEPEs-, (Δ = -1.26; P = 0.020), DHA- (Δ = -0.24; P = 0.004), and HDoHEs- (Δ = -2.61; P = 0.005) derived oxylipins. On the other hand, the HIIT+EMS group decreased plasma levels of DGLA- (Δ = -0.10; P = 0.032), omega-3- (Δ = -0.18; P = 0.009), EPA- (Δ = -0.18; P = 0.024), HEPEs (Δ = -0.96; P = 0.035), DHA- (Δ = -0.19; P = 0.003), and HDoHEs- (Δ = -1.91; P = 0.005) derived oxylipins. However, all these effects were not significant differences from the control group (P > 0.05; **Figure 1E-O**).

Regarding individual oxylipins changes, the interaction network pathway analysis showed that the control group increased plasma levels of TXB2 (Δ = 0.28; P = 0.04), and decreased plasma levels of (i.e., 8-HDoHE, and 11-HDoHE (all $\Delta \leq$ -0.13, P $\leq$ 0.037; **Figure S2A)**. In addition, the PAR group showed significantly decreased plasma levels of EPA (Δ = -0.19; P = 0.02) and EPA-derived oxylipins (i.e., HEPEs and 17,18-DiHETE; all $\Delta \leq$ -0.11, P $\leq$ 0.042), as well as the oxylipins from the HDoHEs pathway (all $\Delta \leq$ -0.18, P $\leq$ 0.017; **Figure S2B**). The HIIT group showed significantly decreased plasma levels of LA-derived oxylipins (e.g., HODEs all $\Delta \leq$ -0.15, P $\leq$ 0.042), DGLA and its derived oxylipins (i.e., HETrEs; all $\Delta \leq$ -0.13, P $\leq$ 0.034), AA and its derived oxylipins (e.g., HETEs, lipoxins; all $\Delta \leq$ -0.07, P $\leq$ 0.040), EPA and its derived oxylipins (i.e., HEPEs and PGE_3; all $\Delta \leq$ -0.22, P $\leq$ 0.041), and DHA-derived oxylipins (all $\Delta \leq$ -0.21, P $\leq$ 0.048: **Figure S2C**). Lastly, the interaction network pathway analysis of the HIIT+EMS group showed that plasma levels of AA-derived oxylipins (i.e., HETEs; all $\Delta \leq$ -0.13, P $\leq$ 0.033), EPA (Δ = -0.23, P = 0.001), HEPEs (all $\Delta \leq$ -0.17, P $\leq$ 0.042), DHA (Δ = -0.11, P $\leq$ 0.007), and HDoHEs (all $\Delta \leq$ -0.11, P $\leq$ 0.034) significantly decreased after the 12-week exercise intervention (**Figure S2D**). However, none of above-mentioned decrements in plasma levels of oxylipins were

statistically different from the control group after post-hoc Bonferroni corrections (P > 0.05; **Figure 2**). Only plasma levels of the LA-derived oxylipin 9,10-EpOME significantly increased in the HIIT+EMS group (Δ = 0.02) in comparison to the PAR group (Δ = -0.08) after post-hoc Bonferroni correction (P = 0.016; **Figure 2**). Additionally, none of the omega 6/omega 3 oxylipins ratio were significantly affected by the different interventions (all P > 0.05; **Table S3**). Changes in individual oxylipins could be found in **Table S4**.

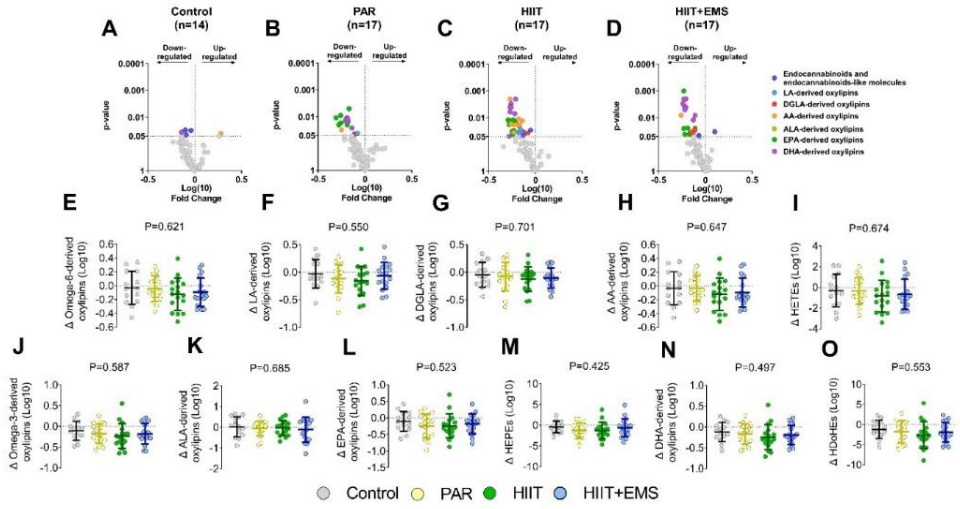

Figure 1. Effect of different exercise training modalities on plasma levels of oxylipins and endocannabinoids.

Panels A-C: The X-axis shows the mean log10 fold change (post minus baseline values) of the area peak ratio of each parameter, while the Y-axis shows the P values obtained from paired t-test. Colored dots mean statistically significant downregulated/upregulated molecules after the intervention, grey dots were not significant after the intervention. Panels E-O: For the analyses, the sum of omega-6 (E) LA-derived (F), DGLA-derived (G), AA-derived (H), HETEs (I), omega-3 (J), ALA-derived (K), EPA-derived (L), HEPEs (M), DHA-derived (N), and HDoHEs (O) oxylipins were calculated. Δ was calculated as post minus baseline values of the area peak ratio for each oxylipin group. P values obtained from analyses of covariance (ANCOVA) adjusting for baseline values. Bars represent mean and standard deviation. *Abbreviations*: AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DHA, Docosahexaenoic acid; EPA, eicosapentaenoic acid; HDoHEs, hydroxy-docosahexaenoic acids; HEPEs, hydroxy-eicosapentaenoic acids; HETEs, hydroxy- arachidonic acids; HIIT, high-intensity interval training group; HIIT+EMS, high-intensity interval training + whole-body electromyostimulation group; LA, linoleic acid.; PAR, physical activity recommendations group.

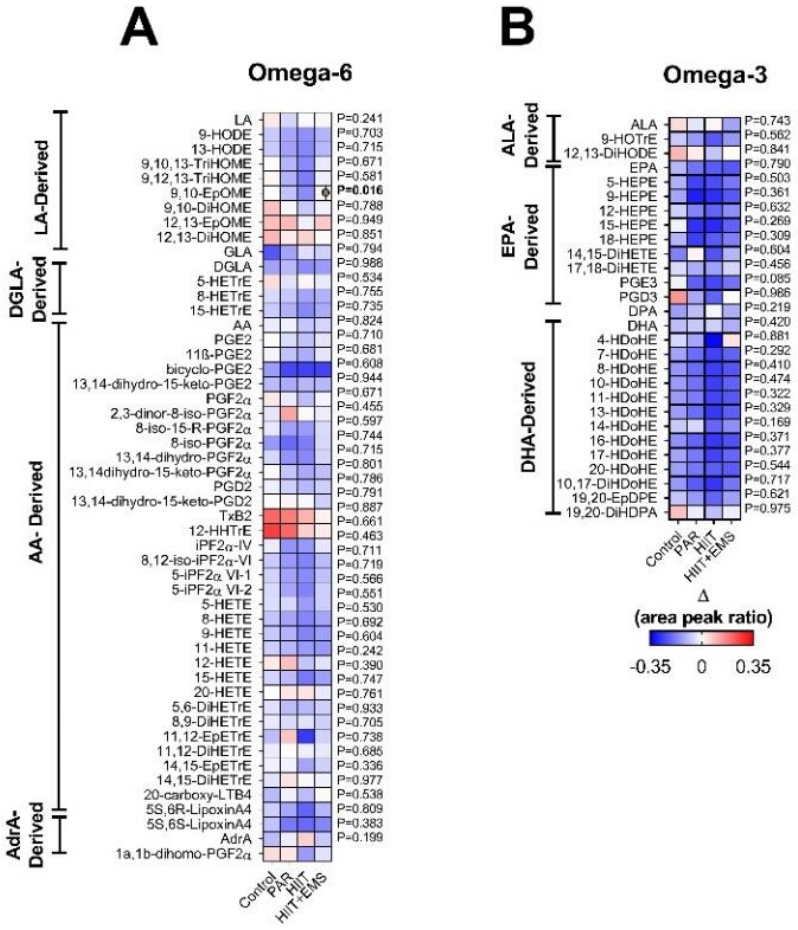

Figure 2. Effect of different exercise training modalities on plasma levels of oxylipins.

Each square represents the change after the intervention (Δ), which was calculated as post minus baseline values of the area peak ratio for each oxylipin. Blue squares mean a decrease, whereas red squares indicate an increase after the intervention, respectively. P values obtained from analyses of covariance (ANCOVA) adjusting for baseline values. Φ Symbol means significant differences between HIIT+EMS and PAR groups after post-hoc Bonferroni correction. *Abbreviations*: HIIT, high-intensity interval training group; HIIT+EMS, high-intensity interval training + whole-body electromyostimulation group; PAR, physical activity recommendations group.

Effect of different exercise training modalities on plasma levels of endocannabinoids and endocannabinoids-like molecules

After the 12-week intervention, the plasma levels eCBs and eCBs-like molecules did not change in the PAR group (all P > 0.05; **Figure 3**). Contrarily, the HIIT group decreased plasma levels of the 2-AG (Δ = -0.13; P = 0.045) and LEA (Δ = -0.05; P = 0.029), whereas the HIIT+EMS group decreased plasma levels of the AEA (Δ = -0.07; P = 0.046), and increased plasma levels of PDEA (Δ = 0.10; P = 0.033). Of note that, after post-hoc Bonferroni correction, only plasma levels of AEA and OEA significantly increased in the PAR group compared to the control group (AEA: Δ = 0.05 vs. -0.09, P = 0.014; and OEA: Δ = -0.12 vs. 0.013, P = 0.049; **Figure 3)**.

Lastly, no significant differences between groups were observed in oxylipins, eCBs eCBs-like molecules changes after correcting for FDR (data not shown). We also observed similar results when the analyses were performed in men and women separately or when they were adjusted for dietary intake of PUFA, fish, and nuts (data not shown).

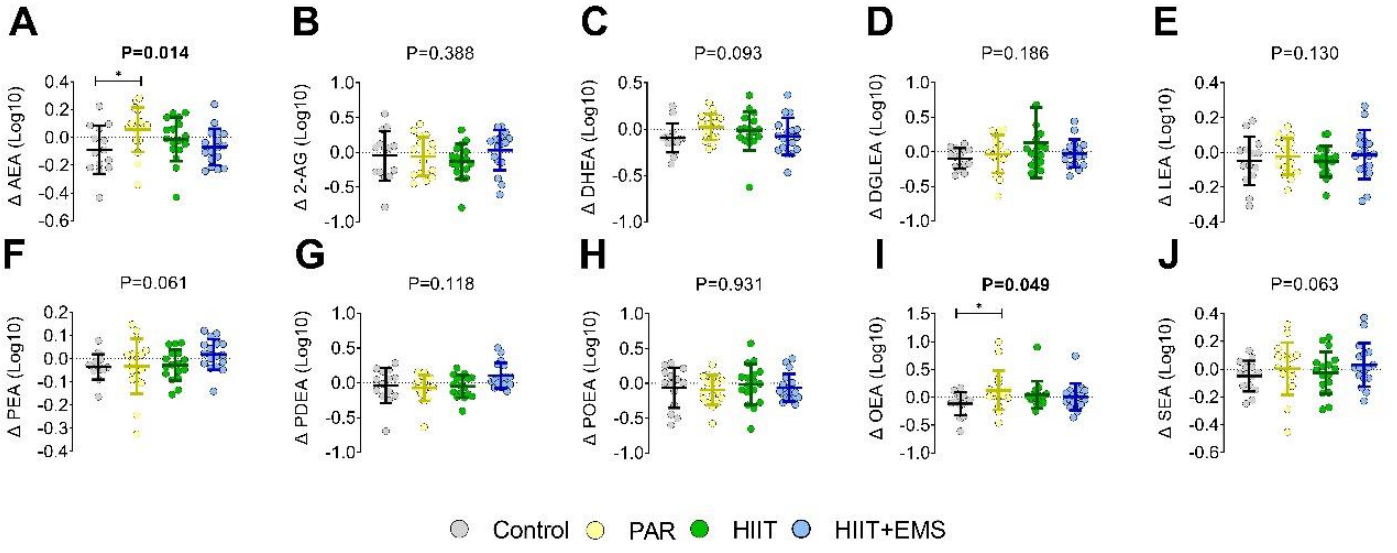

Figure 3. Effect of different exercise training modalities on plasma levels of endocannabinoids (A, B) and endocannabinoids-like molecules (C-J).

Δ was calculated as post minus baseline concentration values for each endocannabinoid and analogue. P value obtained from analyses of covariance (ANCOVA) adjusting for baseline values. Bars represent mean and standard deviation. * Symbol means significant differences between groups after post-hoc Bonferroni correction. Abbreviations: 2-AG, 2-arachidonylglycerol; AEA, anandamide; DGLEA, dihomo-gamma-linolenoyl ethanolamide; DHEA, docosahexaenoyl ethanolamide; HIIT, high-intensity interval training group; HIIT+EMS, high-intensity interval training + whole-body electromyostimulation group; LEA, linoleoyl ethanolamide; OEA, oleoyl ethanolamine; PAR, physical activity recommendations group; PDEA, pentadecanoyl ethanolamide; PEA, palmitoyl ethanolamide; POEA, palmitoleoyl ethanolamide; SEA, stearoyl ethanolamide.

Discussion

The 12-week exercise intervention did not modify plasma levels of omega-6 and omega-3 oxylipins, eCBs, and eCBs-like molecules in middle-aged sedentary adults. Indeed, no statistically significant differences were observed in the exercise training groups (i.e., PAR, HIIT, and HIIT+EMS) compared with the control group in plasma levels of omega-6 and omega-3 oxylipins, eCBs and eCBs-like molecules. However, based on the trends observed in plasma levels of oxylipins in the exercise groups (i.e., decreased plasma levels), we can speculate that exercise may play a potential role in the regulation of the oxylipins metabolic pathways. Additionally, as previously published, this exercise intervention was effective in the reduction of adiposity, improvement of the cardiometabolic profile, and physical fitness [27,32,33]. The present results suggest that these exercise-related benefits might be independent of changes in plasma levels of oxylipins, eCBs, and eCBs-like molecules.

Exercise training tend to decrease plasma levels of oxylipins

Our data show that 12 weeks of concurrent training, high-intensity interval training, and high-intensity interval training plus whole-body electromyostimulation showed a trend towards a decrease in plasma levels of oxylipins. Similarly, both 2-weeks and 145 days of triathlon trainings decreased the levels of omega-6 and omega-3 derived isoprostanes and prostaglandins in urine of triathletes [17,18]. These results may partially concur with our results, since we observed that HIIT and HIIT+EMS group plasma levels of isoprostanes and prostaglandins decreased, suggesting that exercise intensity may have a role in the regulation of these metabolites since no changes were observed in the PAR group. Indeed, only HIIT and HIIT+EMS decreased the plasma levels of AA-derived oxylipins, which are the main intermediates of the AA metabolic pathway, whereas PAR group did not show any differences. However, it is important to be aware of the physiological differences between triathletes and middle-aged sedentary adults. Interestingly, an acute exercise performed at low intensities displayed lower fold-changes in the levels of oxylipins than prolonged and high-intensity acute exercise [10], which suggests that exercise duration and intensity play an important role in the modulation of the plasma concentration of these metabolites.

The mechanisms by which exercise might decrease plasma levels of oxylipins are yet to be unravelled. The synthesis of oxylipins production is initiated with the release of PUFAs

from the cell-membrane phospholipids by the action of phospholipase A2 [34,35]. In this context, exercise training can decrease PUFA content in the cell membrane phospholipids of white adipose tissue and triacylglycerols [36,37] . This could partially explain the decrease in plasma levels of oxylipins observed, since exercise seems to lead to lower levels of their precursors in tissues. The potential consequences of these decrements in plasma levels of oxylipins are still being investigated but may include the regulation of body composition, inflammation, cardiometabolic health, vascular function, oxidative stress, immune system, and kidney function [14,38-40]. Even though our results may provide a new insight into the potential benefits of exercise training in health, more well-powered studies involving long-term exercise training interventions are required to establish the physiological functions of these changes in human physiology.

Exercise training does not modify plasma levels of endocannabinoids and endocannabinoids-like molecules

The different exercise interventions did not alter the plasma levels of eCBs and eCBs-like molecules with statistical significance in middle-aged sedentary adults. These results may partially concur with what has been described in a recent review, which showed that gene expression of the classical (i.e., CB1 and CB2) and novel cannabinoid receptors (i.e., G-protein receptors) in skeletal muscle as was not modified by chronic aerobic, resistance and concurrent exercise training [9]. Notwithstanding, the evidence regarding the effect of chronic exercise training on plasma levels of eCBs is still scarce. In women with fibromyalgia, 15 weeks of resistance exercise training increased AEA and decreased SEA circulating levels [41], whereas 12-week of aerobic exercise training decreased AEA circulating levels in migraine patients [42]. In line with these findings, our data showed a slight increase in plasma levels of AEA and OEA only in the PAR group compared with the control group. Previous studies have shown that the acute increase in AEA levels is dependent on the exercise intensity, i.e., only increasing after acute moderate- but not vigorous-intensity exercise [15,16] Therefore, since we did not observe changes in plasma levels of AEA in the more intense exercise groups (i.e., HIIT and HIIT+EMS), our results may suggest that chronic exercise plasma could have a potential role in the modulation of plasma levels of eCBs. The changes in plasma levels of AEA could be potentially related to the positive central effects of moderate-intensity exercise, such as analgesia or the *runner-high* effects [9,16]. Unfortunately,

our study did not investigate the effects of the exercise intervention on central effects or pain-related outcomes. Finally, although the results obtained from different studies should be compared with caution due to the methodological differences, further studies with similar exercise protocols at different intensities are needed to confirm these preliminary findings.

Limitations

This work is not without limitations. The reduced sample size and the large standard deviation of the changes in each study group has likely underpowered the present work for detecting statistical differences between exercise training groups. Probably, increasing the sample size may lead to statistical differences between groups. The study population included middle-aged sedentary adults, which does not allow for potential extrapolation of the findings to older, younger, or unhealthy populations. However, to the best of our knowledge, this is the first chronic exercise intervention study performed on this topic so far.

Conclusion

In summary, our study finds out that a 12-week exercise training intervention does not modify plasma levels of omega-6 and omega-3 oxylipins, eCBs and eCBs -like molecules in middle-aged sedentary adults. Although plasma levels of omega-6 and omega-3 oxylipins tended to decrease, no statistically significant differences were observed between the exercise training groups (i.e., PAR, HIIT, and HIIT+EMS) compared to the control group. These findings shed new evidence on the potential role of exercise training on the levels of these lipids and warrant further well-designed studies to determine the potential contribution of these metabolites to human health.

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SUPPLEMENTARY RESULTS

Table S1. List of oxylipins, endocannabinoids and endocannabinoids-like molecules analyzed, including relative standard deviations (RSDs) observed in the quality control (QC) samples.

Abbreviation	Name (International Union of Pure and Applied Chemistry, IUPAC)	ChEBI ID	RSD in QC samples
Omega-6 PUFA and oxylipins			
LA	9Z,12Z-octadecadienoic acid	17351	8.0
9-HODE	(±)-9-hydroxy-10E,12Z-octadecadienoic acid	72651	13.3
13-HODE	(±)-13-hydroxy-9Z,11E-octadecadienoic acid	72639	11.3
9,10,13-TriHOME	9,10,13-trihydroxy-11E-octadecenoic acid	34499	27.2
9,12,13-TriHOME	9,12,13-trihydroxy-10E-octadecenoic acid	34506	27.1
9,10-EpOME	9,10-epoxy-12Z-octadecenoic acid	34494	12.9
9,10-DiHOME	9,10-dihydroxy-12Z-octadecenoic acid	72663	9.8
12,13-EpOME	(±)-12(13)-epoxy-9Z-octadecenoic acid	38229	8.9
12,13-DiHOME	12,13-dihydroxy-9Z-octadecenoic acid	72665	9.2
GLA	6Z,9Z,12Z-octadecatrienoic acid	28661	16.7
DGLA	8Z,11Z,14Z-eicosatrienoic acid	53486	22.2
5-HETrE	5S-hydroxy-6E,8Z,11Z-eicosatrienoic acid	88359	17.9
8-HETrE	8S-hydroxy-9E,11Z,14Z-eicosatrienoic acid	140473	13.0
15-HETrE	15S-hydroxy-8Z,11Z,13E-eicosatrienoic acid	88348	8.4
AA	5Z,8Z,11Z,14Z-eicosatetraenoic acid	15843	10.5
PGE2	9-oxo-11R,15S-dihydroxy-5Z,13E-prostadienoic acid	15551	10.6
11β-PGE2	9-oxo-11β,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid	89581	9.5
bicyclo-PGE2	11-deoxy-13,14-dihydro-15-keto-11β,16. xi.-cycloprostaglandin E2	89568	10.2
13,14-dihydro-15-keto-PGE2	9,15-dioxo-11α-hydroxy-prost-5Z-en-1-oic acid	15550	14.9
PGF2α	9α,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid	15553	11.5
2,3-dinor-8-iso-PGF2α	9α,11α,15S-trihydroxy-2,3-dinor-(8β)-prosta-5Z,13E-dien-1-oic acid	NA	30.8
8-iso-15-R-PGF2α	9α,11α,15R-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid	NA	8.0

8-iso-PGF2α	9α,11α,15S-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid	34509	11.9
13,14-dihydro-PGF2α	9α,11α,15S-trihydroxy-prost-5Z-en-1-oic acid	88346	10.4
13,14-dihydro-15-keto-PGF2α	9α,11α-dihydroxy-15-oxo-prost-5Z-en-1-oic acid	63976	27.0
PGD2	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic acid	15555	20.7
13,14-dihydro-15-keto-PGD2	9α-hydroxy-11,15-dioxo-prost-5Z-en-1-oic acid	72603	28.3
TXB2	9S,11,15S-trihydroxy-thromboxa-5Z,13E-dien-1-oic acid	28728	19.9
12-HHTrE	12-hydroxy-5Z,8E,10E-heptadecatrienoic acid	63977	7.6
iPF2α-IV	(8S)-10-[(1R,2S,3S,5R)-3,5-Dihydroxy-2-pentylcyclopentyl]-8-hydroxydeca-5,9-dienoic acid	NA	8.4
8,12-iso-iPF2α-VI	(12α)-5,9α,11α-trihydroxy-prosta-6E,14Z-dien-1-oic acid	NA	9.8
5-iPF2α VI	(8β)-5,9α,11α-trihydroxy-prosta-6E,14Z-dien-1-oic acid	140933	11.1
5-HETE	5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid	28209	5.3
8-HETE	8-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid	34486	8.0
9-HETE	9-hydroxy-5Z,7E,11Z,14Z-eicosatetraenoic acid	72786	13.1
11-HETE	11-hydroxy-5Z,8Z,12E,14Z-eicosatetraenoic acid	72606	16.1
12-HETE	12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid	19138	13.5
15-HETE	15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid	64017	11.7
20-HETE	20-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	34306	12.4
5,6-DiHETrE	5,6-dihydroxy-8Z,11Z,14Z-eicosatrienoic acid	63974	13.7
8,9-DiHETrE	8,9-dihydroxy-5Z,11Z,14Z-eicosatrienoic acid	63970	17.4
11,12-EpETrE	11,12-epoxy-5Z,8Z,14Z-eicosatrienoic acid	34130	19.1
11,12-DiHETrE	11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid	63969	15.5
14,15-EpETrE	14,15-epoxy-5Z,8Z,11Z-eicosatrienoic acid	34157	16.8
14,15-DiHETrE	14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid	63966	11.2
20-carboxy-LTB4	5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraene-1,20-dioic acid	27562	30.4
5S,6R-LipoxinA4	5S,6R,15S-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid	6498	24.5
5S,6S-LipoxinA4	5S,6S,15S-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid	63990	23.3
AdrA	7Z,10Z,13Z,16Z-docosatetraenoic acid	53487	26.5
1a,1b-dihomo-PGF2α	1a,1b-dihomo-9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid	NA	17.7

Omega-3 PUFA and oxylipins

ALA	9Z,12Z,15Z-octadecatrienoic acid	27432	18.5
9-HOTrE	9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid	80447	14.0
12,13-DiHODE	(±)-12,13-dihydroxy-9Z,15Z-octadecadienoic acid	88461	26.5
EPA	5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid	28364	14.6
5-HEPE	(±)-5-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid	72801	11.5
9-HEPE	(±)-9-hydroxy-5Z,7E,11Z,14Z,17Z-eicosapentaenoic acid	89570	8.1
12-HEPE	(±)-12-hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid	72645	11.0
15-HEPE	(±)-15-hydroxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid	72627	11.9
18-HEPE	(±)-18-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid	72802	16.0
14,15-DiHETE	(±)-14,15-dihydroxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid	88459	22.7
17,18-DiHETE	(±)-17,18-dihydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	88349	29.3
PGE3	9-oxo-11α,15S-dihydroxy-prosta-5Z,13E,17Z-trien-1-oic acid	28031	25.4
PGD3	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E,17Z-trien-1-oic acid	34939	26.9
DPA	7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid	61204	16.8
DHA	4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid	28125	11.4
4-HDoHE	(±)-4-hydroxy-5E,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid	72624	13.4
7-HDoHE	(+/-)-7-hydroxy-4Z,8E,10Z,13Z,16Z,19Z-docosahexaenoic acid	72623	9.6
8-HDoHE	(±)-8-hydroxy-4Z,6E,10Z,13Z,16Z,19Z-docosahexaenoic acid	72610	13.7
10-HDoHE	(+/-)-10-hydroxy-4Z,7Z,11E,13Z,16Z,19Z-docosahexaenoic acid	72640	11.7
11-HDoHE	(±)-11-hydroxy-4Z,7Z,9E,13Z,16Z,19Z-docosahexaenoic acid	72794	16.0
13-HDoHE	(±)-13-hydroxy-4Z,7Z,10Z,14E,16Z,19Z-docosahexaenoic acid	72608	14.0
14-HDoHE	(±)-14-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid	72647	13.7
16-HDoHE	(±)-16-hydroxy-4Z,7Z,10Z,13Z,17E,19Z-docosahexaenoic acid	72613	14.4
17-HDoHE	(±)-17-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid	72637	14.9
20-HDoHE	(±)-20-hydroxy-4Z,7Z,10Z,13Z,16Z,18E-docosahexaenoic acid	72615	15.8
10,17-DiHDoHE	10,17-dihydroxy-4Z,7Z,11E,13Z,15E,19Z-docosahexaenoic acid	138653	27.2
19,20-EpDPE	(±)-19(20)-epoxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid	72653	10.6
19,20-DiHDPA	(±)-19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid	72657	10.6

Endocannabinoids and endocannabinoids-like molecules			
AEA	N-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-ethanolamine	2700	15
2-AG	2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycerol	52392	6.3
DHEA	N-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-ethanolamine	85252	18.1
DGLEA	N-(8Z,11Z,14Z-eicosatrienoyl)-ethanolamine	34488	23.5
LEA	N-(9Z,12Z-octadecadienoyl)-ethanolamine	64032	11.4
PEA	N-hexadecanoyl-ethanolamine	71464	9.1
PDEA	N-(Pentadecanoyl)-ethanolamine	N/A	26.4
POEA	N-(9Z-hexadecenoyl)-ethanolamine	71465	35.5
OEA	N-(9Z-octadecenoyl)-ethanolamine	71466	13.4
SEA	N-(Octadecanoyl)-ethanolamine	85299	10.5

Abbreviations: ChEBI: Chemical Entities of Biological Interest, QC: quality control,

RSD: relative standard error.

Table S2. List of internal standards used in the LC-MS/MS method.

Abbreviation	Name (International Union of Pure and Applied Chemistry, IUPAC)
Arachidonic Acid-d8 (C20:4-w6-d8)	5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid-d8
Docosahexaenoic Acid-d5 (C22:6-w3-d5)	4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosahexaenoic acid-d5
Linoleic Acid-d4 (C18:2-w6-d4)	9Z,12Z-octadecadienoic acid-d4
d11-5-iPF2α-VI	(8β)-5,9α,11α-trihydroxy-prosta-6*E*,14*Z*-dien-1-oic acid-d11
d4-8-iso-PGE2	9-oxo-11α,15*S*-dihydroxy-(8β)-prosta-5*Z*,13*E*-dien-1-oic acid-d4
d4-8-iso-PGF2α	9α,11α,15*S*-trihydroxy-(8β)-prosta-5*Z*,13*E*-dien-1-oic acid-d4
d4-PGD2	9α,15*S*-dihydroxy-11-oxo-prosta-5*Z*,13*E*-dien-1-oic acid-d4
d4-PGF2α	9*S*,11*R*,15*S*-trihydroxy-5*Z*,13*E*-prostadienoic acid-d4
d9-PGE2	9-oxo-11*R*,15*S*-dihydroxy-5*Z*,13*E*-prostadienoic acid-d9
d4-iPF2α -IV	(8*S*)-10-[(1*R*,2*S*,3*S*,5*R*)-3,5-Dihydroxy-2-pentylcyclopentyl]-8-hydroxydeca-5,9-dienoic acid-d4
d11-8,12-iso-iPF2α -VI	(12α)-5,9α,11α-trihydroxy-prosta-6*E*,14*Z*-dien-1-oic acid-d11
d17-10-Nitrooleate	10-nitro,9Z,12*Z*-octadecadienoic acid-d17
d11-14,15-DiHETrE	14,15-dihydroxy-5*Z*,8*Z*,11*Z*-eicosatrienoic acid-d11
d4-9-HODE	9-hydroxy-10*E*,12*Z*-octadecadienoic acid-d4
d4-LTB4	5*S*,12*R*-dihydroxy-6*Z*,8*E*,10*E*,14*Z*-eicosatetraene-1,20-dioic acid-d4
d4-TXB2	9*S*,11,15*S*-trihydroxy-thromboxa-5*Z*,13*E*-dien-1-oic acid-d4
d6-20-HETE	20-hydroxy-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid-d6
d8-12-HETE	12-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid-d8
d8-5-HETE	5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid-d8
d4-12,13-DiHOME	12,13-dihydroxy-9*Z*-octadecenoic acid -d4
d8-2-AG	2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycerol-d8
d8-AEA	N-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-ethanolamine-d8
d4-COR	11β,17,21-trihydroxypregn-4-ene-3,20-dione-d4
d4-DHEA	N-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-ethanolamine-d4
d4-LEA	N-(9Z,12Z-octadecadienoyl)-ethanolamine-d4
d4-OEA	N-(9Z-octadecenoyl)-ethanolamine-d4
d4-PEA	N-hexadecanoyl-ethanolamine-d4
d3-SEA	N-(Octadecanoyl)-ethanolamine-d3

Table S3. Effect of different exercise training modalities on PUFA/Oxylipins ratio.

	Control		**PAR**		**HIIT**		**HIIT+EMS**		
	Δ	P	Δ	P	Δ	P	Δ	P	P ANCOVA
AA/HETEs ratio	0.00	0.988	0.00	0.692	0.00	0.684	0.00	0.867	0.357
EPA/HEPEs ratio	-0.01	0.541	-0.01	0.162	-0.02	0.187	-0.34	**0.018**	0.205
DHA/HDoHEs ratio	0.00	0.744	0.01	0.064	0.02	**0.005**	0.01	0.080	0.742

Data are presented as Δ, which was calculated as post minus baseline values of the ratios. P values of each intervention group were obtained from paired t-test. P values obtained from analyses of covariance (ANCOVA) adjusted for baseline values. *Abbreviations*: HIIT, high-intensity interval training group; HIIT+EMS, high-intensity interval training + whole-body electromyostimulation group; PAR, physical activity recommendations group.

Table S4. Effect of different exercise training modalities on fasting levels of oxylipins.

	Control		**PAR**		**PAR**		**HIIT+EMS**	
	Δ	SD	Δ	SD	Δ	SD	Δ	SD
Omega-6 PUFA and oxylipins								
LA	0.03	0.32	-0.06	0.22	-0.01	0.16	0.01	0.28
9-HODE	-0.07	0.26	-0.12	0.26	-0.15	0.27	-0.10	0.26
13-HODE	-0.07	0.28	-0.13	0.28	-0.16	0.29	-0.10	0.27
9,10,13-TriHOME	0.00	0.34	-0.10	0.33	-0.16	0.31	-0.03	0.28
9,12,13-TriHOME	0.01	0.35	-0.11	0.35	-0.18	0.32	-0.02	0.29
9,10-EpOME	0.00	0.20	-0.08	0.15	-0.16	0.27	0.02	0.20
9,10-DiHOME	0.08	0.35	-0.01	0.31	-0.07	0.27	-0.02	0.25
12,13-EpOME	0.09	0.19	0.09	0.30	-0.02	0.20	0.07	0.20
12,13-DiHOME	0.10	0.27	0.03	0.24	0.06	0.18	0.00	0.16
GLA	-0.23	0.46	-0.12	0.24	-0.04	0.40	-0.06	0.38
DGLA	-0.13	0.27	-0.10	0.25	-0.16	0.27	-0.14	0.25
5-HETrE	0.05	0.42	-0.04	0.41	0.01	0.36	-0.03	0.29
8-HETrE	-0.05	0.22	-0.07	0.25	-0.13	0.22	-0.11	0.18
15-HETrE	-0.07	0.29	-0.11	0.32	-0.16	0.28	-0.12	0.26
AA	-0.03	0.15	-0.02	0.11	-0.08	0.14	-0.06	0.15
PGE2	0.00	0.23	-0.05	0.25	-0.08	0.26	-0.03	0.20
11β-PGE2	-0.02	0.26	-0.08	0.30	-0.11	0.30	-0.04	0.21
bicyclo-PGE2	-0.13	0.28	-0.25	0.44	-0.26	0.40	-0.26	0.35
13,14-dihydro-15-keto-PGE2	-0.09	0.30	-0.12	0.36	-0.10	0.34	-0.10	0.28
PGF2α	0.03	0.32	-0.03	0.23	-0.09	0.28	-0.01	0.28
2,3-dinor-8-iso-PGF2α	-0.03	0.29	0.12	0.30	-0.01	0.30	-0.02	0.20
8-iso-15-R-PGF2α	-0.03	0.27	-0.13	0.30	-0.14	0.32	-0.05	0.24
8-iso-PGF2α	-0.14	0.33	-0.21	0.43	-0.17	0.49	-0.05	0.19
13,14-dihydro-PGF2a	-0.05	0.35	-0.15	0.42	-0.16	0.38	-0.06	0.30
13,14-dihydro-15-keto-PGF2α	0.00	0.38	-0.08	0.34	-0.12	0.33	-0.09	0.33
PGD2	-0.02	0.25	-0.06	0.28	-0.10	0.28	-0.06	0.23
13,14-dihydro-15-keto-PGD2	0.00	0.21	0.01	0.28	-0.01	0.25	-0.06	0.23
TXB2	0.21	0.49	0.18	0.50	0.10	0.50	0.02	0.68
12-HHTrE	0.26	0.45	0.21	0.57	0.06	0.39	0.02	0.69
iPF2α-IV	-0.02	0.30	-0.15	0.32	-0.14	0.32	-0.03	0.26
8,12-iso-iPF2α-VI	-0.07	0.29	-0.14	0.28	-0.17	0.31	-0.09	0.24
5-iPF2α VI-1	-0.06	0.28	-0.13	0.28	-0.16	0.31	-0.09	0.23
5-iPF2α VI-2	-0.05	0.29	-0.12	0.28	-0.17	0.30	-0.07	0.22
5-HETE	-0.05	0.20	-0.05	0.20	-0.14	0.20	-0.07	0.16
8-HETE	-0.08	0.27	-0.12	0.26	-0.17	0.26	-0.12	0.24
9-HETE	-0.07	0.27	-0.10	0.29	-0.17	0.27	-0.13	0.23
11-HETE	-0.07	0.28	-0.10	0.28	-0.14	0.27	-0.14	0.24
12-HETE	0.03	0.26	0.09	0.24	-0.08	0.32	-0.04	0.31
15-HETE	-0.06	0.28	-0.09	0.25	-0.18	0.26	-0.13	0.24
20-HETE	0.00	0.28	0.03	0.16	0.04	0.13	-0.03	0.21
5,6-DiHETrE	-0.04	0.23	-0.09	0.23	-0.10	0.23	-0.06	0.20
8,9-DiHETrE	-0.03	0.18	-0.05	0.21	-0.08	0.25	-0.06	0.13
11,12-EpETrE	-0.09	0.37	0.08	0.37	-0.27	0.30	-0.06	0.32
11,12-DiHETrE	-0.02	0.16	0.00	0.19	-0.02	0.12	-0.03	0.13
14,15-EpETrE	-0.02	0.31	-0.02	0.22	-0.12	0.38	-0.06	0.31

14,15-DiHETrE	-0.04	0.16	0.03	0.13	0.01	0.12	-0.02	0.11
20-carboxy-LTB4	-0.10	0.35	-0.08	0.42	-0.09	0.32	0.01	0.36
5S,6R-LipoxinA4	-0.07	0.39	-0.12	0.33	-0.21	0.33	-0.10	0.29
5S,6S-LipoxinA4	-0.13	0.46	-0.16	0.44	-0.23	0.42	-0.13	0.38
AdrA	-0.09	0.30	-0.02	0.22	0.06	0.47	-0.07	0.22
1a,1b-dihomo-PGF2α	0.05	0.34	0.03	0.26	-0.14	0.27	-0.04	0.20
Omega-3 PUFA and oxylipins								
ALA	0.05	0.55	-0.04	0.43	0.01	0.39	-0.13	0.70
9-HOTrE	-0.07	0.26	-0.14	0.27	-0.23	0.36	-0.14	0.31
12,13-DiHODE	0.10	0.22	0.02	0.29	-0.08	0.30	0.00	0.26
EPA	-0.10	0.28	-0.19	0.31	-0.22	0.33	-0.23	0.25
5-HEPE	-0.11	0.31	-0.26	0.40	-0.24	0.36	-0.19	0.31
9-HEPE	-0.12	0.34	-0.31	0.44	-0.28	0.44	-0.23	0.38
12-HEPE	-0.06	0.30	-0.17	0.21	-0.21	0.43	-0.16	0.36
15-HEPE	-0.02	0.31	-0.28	0.45	-0.29	0.42	-0.22	0.40
18-HEPE	-0.10	0.29	-0.26	0.37	-0.23	0.39	-0.17	0.32
14,15-DiHETE	-0.17	0.35	0.01	0.34	-0.24	0.44	-0.10	0.33
17,18-DiHETE	-0.05	0.23	-0.11	0.21	-0.13	0.37	-0.06	0.27
PGE3	-0.02	0.35	-0.22	0.47	-0.25	0.45	-0.19	0.62
PGD3	0.14	0.39	-0.12	0.47	-0.20	0.44	-0.05	0.57
DPA	-0.13	0.24	-0.09	0.23	-0.01	0.35	-0.11	0.18
DHA	-0.09	0.19	-0.07	0.18	-0.06	0.18	-0.11	0.15
4-HDoHE	-0.06	0.21	-0.12	1.08	-0.34	0.85	0.04	0.71
7-HDoHE	-0.13	0.23	-0.20	0.29	-0.26	0.30	-0.21	0.24
8-HDoHE	-0.14	0.23	-0.20	0.30	-0.26	0.32	-0.23	0.24
10-HDoHE	-0.14	0.24	-0.19	0.28	-0.26	0.33	-0.22	0.25
11-HDoHE	-0.14	0.22	-0.18	0.27	-0.26	0.32	-0.23	0.25
13-HDoHE	-0.13	0.26	-0.20	0.29	-0.27	0.33	-0.22	0.27
14-HDoHE	-0.07	0.23	-0.06	0.19	-0.21	0.32	-0.16	0.28
16-HDoHE	-0.15	0.28	-0.21	0.32	-0.27	0.36	-0.24	0.28
17-HDoHE	-0.13	0.25	-0.18	0.27	-0.26	0.31	-0.21	0.26
20-HDoHE	-0.13	0.25	-0.19	0.29	-0.22	0.30	-0.22	0.28
10,17-DiHDoHE	-0.14	0.38	-0.22	0.44	-0.28	0.54	-0.19	0.42
19,20-EpDPE	-0.08	0.21	-0.14	0.23	-0.21	0.23	-0.13	0.19
19,20-DiHDPA	0.08	0.47	-0.03	0.18	-0.09	0.37	-0.02	0.17

Data are presented as Δ (calculated as post minus baseline values) and standard deviation (SD). *Abbreviations*: HIIT, high-intensity interval training group; HIIT+EMS, high-intensity interval training + whole-body electromyostimulation group; PAR, physical activity recommendations group.

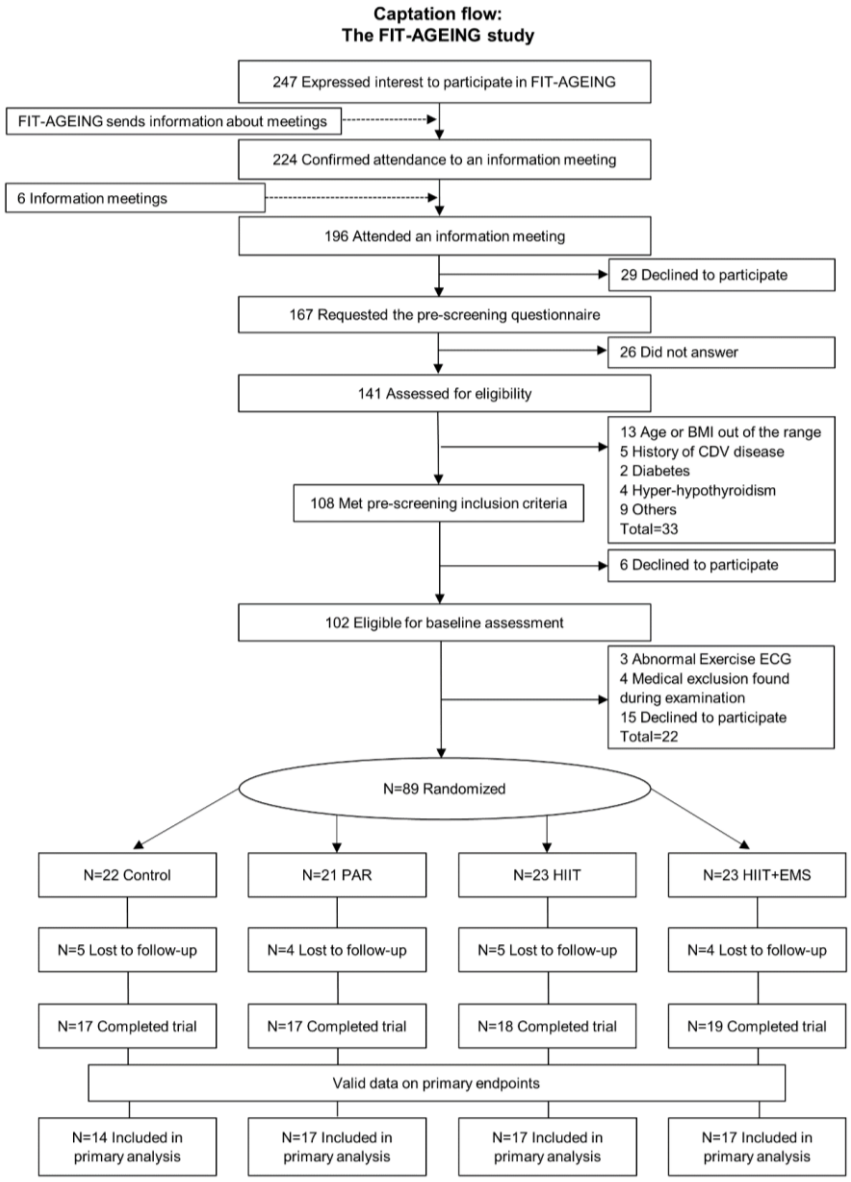

Figure S1. Flow-chart diagram of the study participants. *Abbreviations*: CDV, cardiovascular; ECG, electrocardiogram; HIIT, high-intensity interval training group; HIIT+EMS, whole-body electromyostimulation group; PAR, physical activity recommendations group.

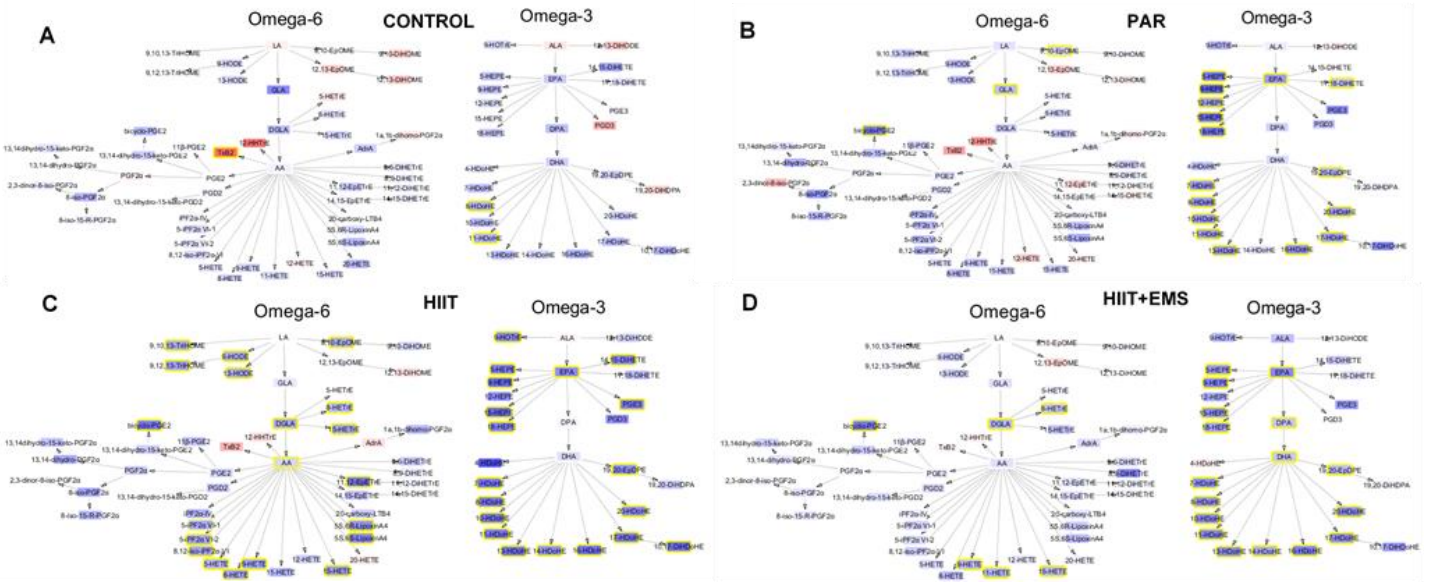

Figure S2. Interaction network pathway analysis of fasting plasma levels of omega-6 and omega-3 oxylipins after 12 weeks of intervention in the control (A), PAR (B), HIIT (C) and HIIT+EMS (D) groups. The networks depict the changes of individual omega-6 and omega-3 oxylipins plasma levels in the PAR group. Blue boxes with yellow frame and red boxes with yellow frame indicate a statistically significant decrease or increase after the intervention, respectively. Blue and red boxes without yellow frame are oxylipins that change without statistical significance. The statistical threshold was set in $P < 0.05$ obtained from the paired t-test. The abbreviations used for each oxylipin are detailed in Table S1. Abbreviations: HIIT, high-intensity interval training group; HIIT+EMS, high-intensity interval training + whole-body electromyostimulation group; PAR, physical activity recommendations group.