Exploration of the endocannabinoid system using metabolomics

Di, X.

Chapter 6

Acute and long-term exercise differently modulate plasma levels of oxylipins, endocannabinoids, and their analogues in young sedentary adults: a randomized controlled-trial

Based on

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EBioMedicine. 2022;85:104313.

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ABSTRACT

Background: Fatty acid-derived lipid mediators including oxylipins, endocannabinoids (eCBs), and their analogues, have emerged as key metabolites in the inflammatory and immune response to physiological stressors.

Methods: This ancillary study was based on data from the ACTIBATE single-center randomized controlled trial (ClinicalTrials.gov ID: NCT02365129). Here, we performed both an acute endurance and resistance exercise sub-studies (n=14 and 17 respectively), and a 24-week supervised exercise intervention, combining endurance and resistance exercise training at moderate-intensity (MOD-EX) or vigorous-intensity (VIG-EX) exercise groups, in young sedentary adults (n=102). Plasma levels of oxylipins, eCBs, and their analogues were measured using liquid chromatography-tandem mass spectrometry.

Findings: Both endurance and resistance exercise increased by +50% the plasma levels of dihomo-γ-linolenic acid and arachidonic acid (AA) omega-6 derived oxylipins, as well as eicosapentaenoic acid and docosahexaenoic acid omega-3 derived after 3 and 120 minutes of the bout of exercise. These exercise modalities also increased the levels of anandamide and eCBs analogues (+25%). After 24-week of supervised exercise, MOD-EX decreased (-12%) plasma levels of AA-derived omega-6 oxylipins, eCBs, and their analogues (-22%) in comparison to the control and VIG-EX groups.

Interpretation: both endurance and resistance exercises acutely increased plasma levels of oxylipins, eCBs, and their analogues, whereas 24 weeks of MOD-EX decreased fasting plasma levels of AA-derived omega-6 oxylipins, eCBs, and their analogues in young, sedentary adults.

1. Introduction

Regular exercise is related to lower odds of mortality and lower prevalence of chronic diseases due to its benefits on cardiovascular, metabolic, and immune health[1,2]. Although the physiological mechanisms of exercise are multifactorial, the health-related benefits of exercise may be partially explained by modulation of the inflammatory and immune status[2,3]. Acute exercise exerts a pro-inflammatory stimulus, whereas long-term exercise training induces anti-inflammatory effects implicated in the health-related benefits of exercise training[2,3]. The physiological mechanisms that underly these effects have primarily been focused on muscle-derived myokines among which interleukin-6 (IL-6), but may also involve additional metabolic tissues and metabolites. Therefore, future research is needed to fully understand the metabolic pathways through which exercise improves inflammation and the immune function[2,4].

Recently, novel lipid mediators, such as oxylipins and endocannabinoids (eCBs), have emerged as key metabolites in the inflammatory and immune response of an organism to infections and injuries[5–7]. Oxylipins are oxidation products of polyunsaturated fatty acids (PUFAs), with either pro-inflammatory or immune impairing functions (i.e., omega-6 derived oxylipins) or anti-inflammatory, pro-resolution and improved immune functions (i.e., omega-3 derived oxylipins)[8–10]. They are the main mediators of the effects of PUFAs on metabolism in humans through their binding to G protein-coupled receptors (GPCRs) or peroxisome proliferator-activate receptors (PPARs)[9]. eCBs, mainly represented by anandamide (AEA) and 2-arachidonyl glycerol (2-AG), activate the G protein-coupled cannabinoid receptors type 1 (CB1R) and 2 (CB2R)[11,12]. Structural analogues of eCBs, such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), have significantly less affinity for CB1R and CB2R[11], but have higher affinity for GPCRs, PPARs or transient receptor potential vanilloid (TRPV)[11]. eCBs and their analogues are implicated in the maintenance of the inflammatory and immune status through the modulation of cytokine production and function of immune cells[11–13].

Previous studies in humans have reported that endurance exercise acutely increases circulating levels of oxylipins and eCBs 120-180 min after exercise[14–18]. The increase in circulating oxylipins is generally smaller after low-exercise intensity or very short exercise

compared to more intense or longer exercise[14–18]. Circulating levels of AEA[19–24] and of the eCBs analogues PEA and OEA[25] also increases in response to acute endurance exercise in humans.

The long-term effect of exercise training on these lipid mediators is poorly understood[3,24,26]. So far, two independent studies shown that long-term (14 and 145 days) endurance exercise decreases urinary oxylipins such as isoprostanes, and plasma prostaglandins in humans[27,28]. On the other hand, we found no evidence of 12 weeks of different training modalities on the plasma levels of oxylipins, eCBs and their analogues in middle-aged adults[29]. This lack of effect might be explained by a low sample size.

Therefore, this study aims to investigate the acute effects of endurance and resistance exercise on plasma levels of oxylipins, eCBs and their analogues, and to study the effects of a 24-week supervised concurrent exercise intervention at moderate and vigorous intensities on the plasma levels of these metabolites in young, sedentary adults.

2. Methods

2.1 Participants

A total of 145 young sedentary Caucasian male and female adults between 18 and 25 years old participated in the ACTIBATE study (ACTivating Brown Adipose Tissue through Exercise; ClinicalTrials.gov ID: NCT02365129; **Figure S1**)[30]. Participants were recruited through social networks, local media, and posters in Granada (Spain). Inclusion criteria included i) reporting to be sedentary (i.e., <20 min/day of moderate-to-vigorous physical activity for <3 days/week), ii) to be non-smoker; and iii) to have stable body weight over the last 3 months. Exclusion criteria included diagnostic of diabetes, hypertension, or other significant chronical medical conditions or medical conditions that can interfere with, or be aggravated by exercise, being pregnant, using medication deemed to affect energy metabolism, and having frequent exposures to cold temperatures.

2.2 Study design

The current report is an ancillary study from the single-centre ACTIBATE randomized controlled trial, of which the detailed study design is described elsewhere[30]. During baseline examinations, a subgroup of participants underwent a sub-study to investigate the acute

effects of endurance (n=14) and resistance (n=17) exercise on plasma levels of oxylipins, eCBs, and their analogues (**Figure 1A**). After baseline examinations, all participants were randomly assigned into three groups using computer-generated simple (unrestricted) randomization by the principal investigator[31], namely, (i) control group (no exercise), (ii) moderate-exercise intensity group (MOD-EX), and (iii) vigorous-exercise intensity group (VIG-EX) (**Figure 1B**). Participants were explicitly informed on the group to which they were assigned. The study was conducted over two consecutive years in 4 different waves (from September 2015 to June 2016, and from September 2016 to June 2017). All participants were instructed not to change their daily routine, physical activity and dietary patterns throughout the study. No important changes were performed in the methodology or outcomes after the beginning of trial.

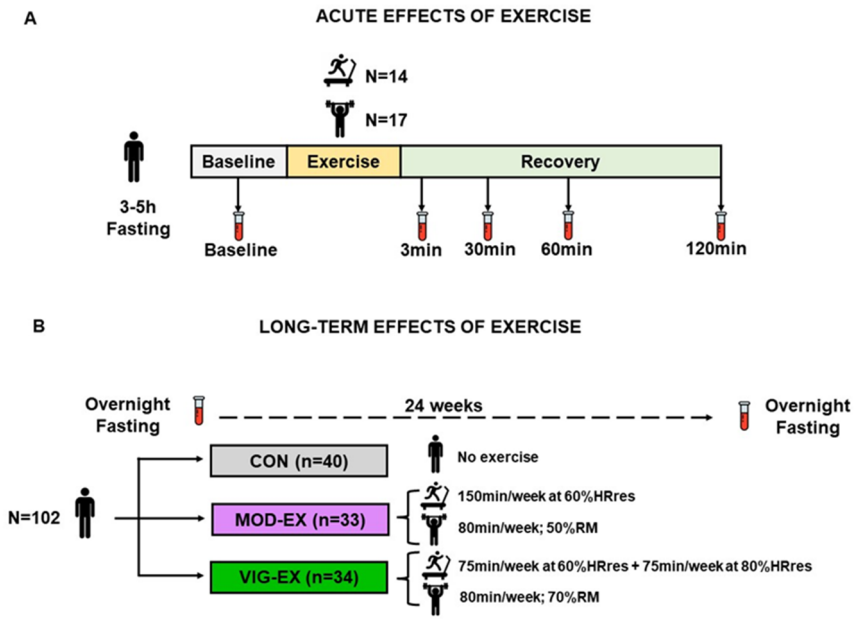

Figure 1. Design of the study investigating the acute (A) and long-term (B) effects of exercise on plasma levels of oxylipins, eCBs and their analogues. Abbreviations: CON, control group; HRes, heart rate reserve; min: minutes: MOD-EX, moderate-intensity exercise group; RM, repetition maximum; VIG-EX, vigorous-intensity exercise group.

2.3 Acute exercise sessions

The exercise session to examine the acute effect consisted of one endurance session and one resistance exercise session in two independent study days. Both were performed in a fasted state (i.e., 3-5h fasting), after avoiding stimulants (e.g., caffeine), and any moderate or vigorous exercise the days prior to the trials (24 h and 48 h, respectively). The endurance exercise trial was a maximum effort test on a treadmill (Pulsar treadmill, H/P/Cosmos Sports & Medical GmbH, Nussdorf-Traunstein, Germany) according to the modified Balke protocol[32]. The resistance exercise trial was comprised of a maximum isometric strength test in leg press, a handgrip strength test and two 1-repetition maximum (1-RM) tests in bench and leg press, as described elsewhere[33]. An extensive description of both exercise sessions can be found in the supplementary methods.

2.4 Long-term exercise intervention

An extensive description of the supervised exercise training program can be found elsewhere[30]. Briefly, the supervised exercise intervention within the ACTIBATE project combined endurance and resistance training, as recommended by the World Health Organization (WHO) guidelines. For 24 weeks, participants attended the research centre 3-4 times per week, 60-90min per session. Both endurance and resistance training were personalized to the participants' physical fitness levels. The intervention was divided into 5 phases of different durations, starting with a familiarization phase of 4 weeks[30].

Participants completed a total of 150 min/week of endurance training (performed in all sessions), performed at 60% of the heart rate reserve (HRres) in the MOD-EX, whereas the VIG-EX performed 75 min/week at 60% HRres and 75 min/week at 80% HRres. A total of 80 min of resistance exercise per week (performed over 2 sessions) was completed by the participants, performed with loads equivalent to the 50%RM in the MOD-EX and to the 70%RM in the VIG-EX. The load for resistance exercises was individually adjusted monthly[30].

Exercise sessions were carried out in groups of 10-16 participants, at the same time of the day during the whole intervention. Attendance of the participants was registered. The adherence to the prescribed intensity for the endurance training was quantified by heart rate monitors (RS800CX, Polar Electro Öy, Kempele, Finland). If participants were unable to

attend a session at the research centre, they were allowed and encouraged to perform unsupervised training sessions elsewhere.

2.5 Blood sample collection

Prior to each acute exercise session, an intravenous catheter was placed in the antecubital vein and blood was collected before each session and 3, 30, 60 and 120 min after the end of each session. For the evaluation of the long-term effects of exercise, blood samples were collected 1-3 weeks before and 3-4 days after the 24-week exercise intervention, in the morning after a 10h overnight fast. Blood samples were obtained with EDTA-coated Vacutainer® Hemogard™ tubes and were immediately centrifuged to obtain plasma. Samples were aliquoted and stored at -80ºC.

2.6 Determination of plasma levels of oxylipins, endocannabinoids and their analogues

Plasma levels of oxylipins, eCBs, and their analogues were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method as described elsewhere[34]. Briefly, plasma samples were prepared with liquid-liquid extraction and analysed using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) connected to a SCIEX QTRAP 6500^{+} mass spectrometer (SCIEX, Framingham, MA, USA). In addition to the study samples, quality control (QC) samples were analysed and used to evaluate the quality of the data and correct for between-batch variations. The relative standard deviations (RSDs) of the peak area ratios were calculated for each analyte present in the QC samples. The sample preparation, LC-MS/MS analysis, and data pre-processing are extensively described in the supplementary material.

The LC-MS/MS protocol enabled the determination of oxylipins derived from the omega-6 PUFAs [i.e., linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), arachidonic acid (AA), and adrenic acid (AdrA)], as well as the oxylipins derived from omega-3 PUFAs [i.e., α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)]. The area peak ratio of all oxylipins derived from omega-6 PUFAs (LA, DGLA, AA, AdrA) and omega-3 PUFAs (ALA, EPA, and DHA) covered by the analytical method were summed from the individual data (see **Table S1** for the oxylipins included in each sum). Additionally, the ratio of omega-6/omega-3 oxylipins was calculated by dividing the sum of omega-6

oxylipins by the sum of omega-3 oxylipins.

This protocol was also used to determine eCBs [i.e., AEA and 2-AG] and their analogues [i.e., docosahexaenoyl ethanolamide (DHEA), dihomo-gamma-linolenoylethanolamide (DGLEA), linoleylethanolamine (LEA), α-linolenoylethanolamide (α-LEA), PEA, pentadecanoylethanolamide (PDEA), palmitoleoylethanolamide (POEA), OEA, stearoylethanolamine (SEA), 2-linoleoylglycerol (2-LG) and 2-oleoylglycerol (2-OG].

The RSD from QC samples of each analyte are listed in **Table S1,** whereas the internal standards used are listed in **Table S2**. In the acute exercise samples, of all the analytes detected, 47 showed a low analytical variability with $QC_{RSD} \leq 15\%$, 21 showed a moderate analytical variability QC_{RSD} between 15% and 30%. In the long-term exercise samples 47 metabolites showed a low analytical variability, and 18 showed a moderate analytical variability in the long-term exercise samples. All metabolites had a fair variability with intraclass correlation coefficients higher than 0.6.

2.7 Anthropometric and body composition measurements

Weight and height were measured barefoot and with light clothing, using a SECA scale and stadiometer (model 799; Electronic Column Scale, Hamburg, Germany), and were used to calculate body mass index (BMI; kg/m^2). 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). Fat mass was also expressed as a percentage of body weight.

2.8 Physical fitness assessment

Physical fitness was assessed during the two sessions described above for the collection of samples to test the acute effects of exercise (all participants underwent a physical fitness evaluation while only 14/17 provided blood samples) (see **supplementary material**).

2.9 Dietary self-reported intake

Dietary self-reported intake was assessed from 24h recalls taken on three separate days before and after the trial, as previously described[35]. Data from the 24h recalls were introduced in the EvalFINUT® software (FINUT, Spain) and the self-reported intake of PUFAs was obtained.

2.10 Basal fat oxidation and maximal fat oxidation

Basal fat oxidation (BFox) was measured by indirect calorimetry (IC) following current recommendations early in the morning[36]. Maximal fat oxidation during exercise (MFO) was assessed by IC using an incremental treadmill test, and following a previously validated methodology[37]. An extensive description of the methodology has been published elsewhere[37].

2.11 Ethics

The study protocol and experimental design were applied in accordance with the last revised ethical guidelines of the Declaration of Helsinki. The study was approved by the Ethics Committee on Human Research of the University of Granada (no. 924) and the Servicio Andaluz de Salud (Centro de Granada, CEI-Granada); all participants gave informed consent.

2.12 Statistical analyses

This study was ancillary to a parent study aimed at determining the effects of a 24-week supervised exercise intervention on brown adipose tissue (BAT) which was originally powered to detect changes in BAT volume and activity. Therefore, no specific power calculation was performed for the current study. For the acute exercise sub-study, we selected a convenience sample of 14 and 17 participants. Based on previous acute exercise studies[26,38,39], this sample size is large enough to detect effects of the exercise intervention on plasma omics parameters.

Descriptive data are expressed as mean ± standard deviation, unless otherwise stated. Firstly, data normality was explored using the Shapiro-Wilk test, visual histograms and Q-Q plots. None of the oxylipins, eCBs and their analogues followed a normal distribution. Thereby, all values were log2-transformed for the analyses of the acute effects and log10-transformed for the analyses of the long-term effects.

The Sex - Time interaction effects on oxylipins, eCBs, and their analogues were analyzed using a mixed model, with “sex” and “time” as fixed effects (data not shown). Since no sex interactions were observed in any of the aforementioned analyses (all $P>0.1$), all analyses were performed after combining men and women data.

The acute effects of endurance and resistance exercise on plasma levels of oxylipins, eCBs, and their analogues were analysed by repeated measures analysis of variance (ANOVA). The fold changes relative to baseline were calculated with the log2-transformed outcomes (i.e., 120 min fold change = log2 area ratio at 120 min minus log2 area ratio at baseline). To account for multiple testing across changes of levels of oxylipins, eCBs, and their analogues, we used the two-stage step-up method of Benjamini-Hochberg false discovery rate (FDR; 0.25) method[40]. Pearson correlation analyses were further performed to evaluate the associations between acute changes in plasma levels of oxylipins, eCBs, and their analogues (i.e., 3- and 120-min fold-changes relative to baseline) and baseline adiposity and physical fitness.

To assess the long-term effects of the exercise intervention on plasma levels of oxylipins, eCBs, and their analogues, no imputation was carried out, and all analysis were performed following the per protocol approach. We firstly performed paired T-tests in each group separately to analyze mean changes from baseline. Next, a delta (Δ=log10 post intervention– log10 baseline values) was calculated for every outcome. Then, analyses of covariance (ANCOVA) were conducted to compare the Δ between groups, including the categorical effects of exercise groups and the corresponding baseline value of each metabolite as a fixed covariate. Bonferroni post-hoc adjustments for multiple comparisons were used to examine differences between groups. Estimated marginal means, 95% confidence interval (CI) and effect sizes (η^2) were also obtained from ANCOVA. Additionally, Pearson correlations were performed to analyze the associations between the Δ of oxylipins, eCBs, and their analogues and the Δ of adiposity and physical fitness parameters, cardiometabolic risk parameters, or dietary self-reported intake (i.e., PUFA intake).

All 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). Significance was set a $P<0.05$.

3. Results

Among the initial 145 participants allocated to any of the three groups for the long-term intervention, 102 participants were included in the analysis (**Figure S1**). 43 participants were excluded from the main analyses as they did not complete the study (i.e., they attended less than 70% of the total training sessions) or they did not have valid measurements for oxylipins, eCBs, and their analogues (**Figure S1**). Phenotypical traits of the participants included in the acute and long-term effects of exercise can be found in **Table 1** and baseline levels of oxylipins, eCBs and their analogues in **Table S3**.

Table 1. Baseline characteristics of the study participants.

	Acute effect of exercise (sub-study)				**Long-term effect of exercise (ancillary study)**					
	Endurance (n=14)		**Resistance (n=17)**		**CON (n=36)**		**MOD-EX (n=33)**		**VIG-EX (n=33)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Demographics										
Age (years)	21.8	2.5	22.4	2.5	22.1	2.1	22.1	2.2	22.2	2.5
Male (n, %)	2	14%	6	35%	16	40%	9	27%	10	30%
Female (n, %)	12	86%	11	64%	24	60%	24	73%	24	70%
Body composition										
BMI (kg/m^2)	24.2	4.0	25.3	4.2	24.3	4.2	25.1	4.3	25.2	4.4
Lean mass (kg)	39.6	7.2	41.8	9.0	41.9	10.8	40.9	7.9	42.0	9.5
Fat mass (kg)	24.1	9.5	26.2	6.7	23.3	7.5	25.6	9.1	25.8	7.9
Fat mass (%)	35.9	10.0	37.1	6.3	34.5	7.2	36.6	8.6	36.5	6.7
VAT mass (g)	326	173	378	160	323	165	359	184	360	185
Physical Fitness										
Handgrip strength (kg)	30.3	5.1	31.6	9.1	31.9	8.2	30.6	7.7	31.0	7.7
RM leg press (kg)	205.2	54.8	210.3	69.6	207.3	74.7	193.3	57.8	200.5	67.4
RM bench press (kg)	28.0	10.0	31.6	13.1	33.2	17.0	28.6	10.5	30.8	13.0
VO_2peak (ml/kg/min)	40.7	7.2	40.0	9.6	42.5	9.2	40.5	6.7	40.2	8.7
Time to exhaustion (s)	806	236	872	219	892	231	938	191	949	179

Data are presented as mean and standard deviation (SD) unless otherwise stated. *Abbreviations*: BMI, body mass index; CON, control group: MOD-EX, moderate-intensity exercise group; RM, repetition maximum; VAT, visceral adipose tissue; VIG-EX, vigorous-intensity exercise group; VO_2, oxygen consumption.

3.1 Endurance and resistance exercise acutely alter plasma levels of oxylipins, endocannabinoids, and their analogues

Overall, we observed that a single session of endurance exercise significantly increased the omega-6/omega-3 oxylipins ratio (P=0.014; **Figure S2A**). Specifically, endurance exercise

significantly decreased LA-derived omega-6 oxylipins (-33.1%; P<0.001; **Figures 2A** and **S3A**), whereas it significantly increased DGLA- and AA-derived omega-6 oxylipins (+48.5% and +59.9%, respectively; both P≤0.011; **Figures 2A** and **S3A**). Moreover, endurance exercise significantly increased EPA-, and DHA-derived omega-3 oxylipins (+88.7% and +56.3%; all P≤0.039; **Figures 2B** and **S3C**), without affecting ALA-derived oxylipins. Endurance exercise also acutely increased AEA (+31%; P=0.009) and decreased 2-AG (-14.6%; P=0.008) (**Figure 2C**), as well as their analogues (i.e., DHEA, DGLEA, LEA, PEA, OEA, and SEA; ~+35%, all P≤0.026; **Figure 2C**).

In contrast, we found that a single session of resistance exercise did not modify the ratio of omega-6/omega-3 oxylipins (P=0.886; **Figure S2B**). However, resistance exercise significantly increased DGLA- and AA- derived omega-6 oxylipins (+23.3% and +86.9%; both P<0.001; **Figure 2D and S3B**), as well as EPA- and DHA-derived omega-3 oxylipins (+52.0% and +31.4%; both P<0.001; **Figures 2E** and **S3D**). Resistance exercise also acutely increased AEA, DGLEA, POEA, and OEA (~+13%, all P≤0.041; **Figure 2F**).

Overall, all significant results persisted after adjusting for false discovery rate, except for the changes in DHA-derived oxylipins after the endurance exercise session and changes in AEA after resistance exercise.

3.2 Long-term moderate-exercise intensity training decreases plasma levels of omega-6 oxylipins and endocannabinoids

After 24-weeks of exercise intervention MOD-EX significantly decreased omega-6-derived oxylipins (-14.9%, P=0.007; **Figure 3D** and **Table S4**). Specifically, MOD-EX significantly decreased the levels of AA-derived oxylipins (-11.7%, P=0.016; **Figures 3G**, **S4A**, and **Table S4**). However, post-hoc comparisons only revealed differences between the MOD-EX group and the control group (P≤0.034; **Figures 3D**, **3G**, **S4A**, and **Table S4**). In addition, MOD-EX tended to decrease LA- and DGLA-derived oxylipins (-18.5%; P=0.097, and -20.3%; P=0.052; **Figures 3E**, F, and **Table S4**). We did not observe changes in omega-3-, ALA-, EPA-, DHA-derived oxylipins or the ratio of omega-6/omega-3 oxylipins after 24-weeks of intervention (all P>0.05; **Figures 3I-M**, **S4B**, and **Table S4**).

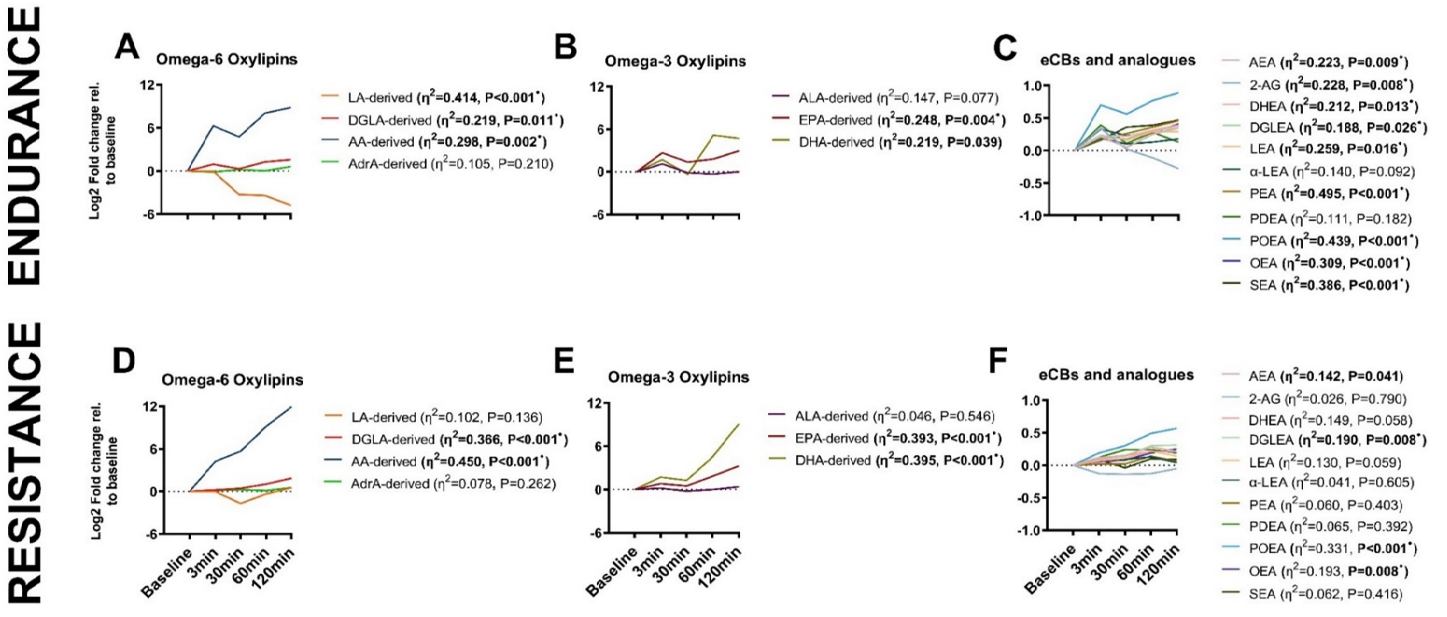

Figure 2. Endurance and resistance exercise acutely increase plasma levels of oxylipins, endocannabinoids, and their analogues. Changes in omega-6 (A, D), omega-3 oxylipins (B, E) and endocannabinoids and their analogues (C, F) after an endurance (A, B, C) and resistance (D, E, F) exercise session. Each line represents the trajectory of the mean log2 fold change relative to baseline of each group of oxylipins or individual endocannabinoid and endocannabinoids analogue. The sum of LA-, DGLA-, AA-, AdrA-, ALA-derived, EPA-, and DHA-derived oxylipins were calculated (A, B, D, E). Depicted η^2 and P values are obtained from repeated measures analyses of variance (ANOVA). • symbol means significant after FDR corrections. ***Abbreviations***: 2-AG, 2-arachidonylglycerol; AA, arachidonic acid; AdrA, adrenic acid; AEA, anandamide; ALA, α-linolenic acid; CON, control group; DGLA, dihomo-γ-linolenic acid; DGLEA, dihomo-gamma-linolenoyl ethanolamide; DHA, Docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamide; ECBs, endocannabinoids; EPA, eicosapentaenoic acid; LA, linoleic acid; LEA, linoleoyl ethanolamide; MOD-EX, moderate intensity exercise group; OEA, oleoyl ethanolamine; PDEA, pentadecanoyl ethanolamide; PEA, palmitoyl ethanolamide; POEA, palmitoleoyl ethanolamide; SEA, stearoyl Ethanolamide; VIG-EX, vigorous intensity exercise group; α-LEA, α-Linolenoyl ethanolamide.

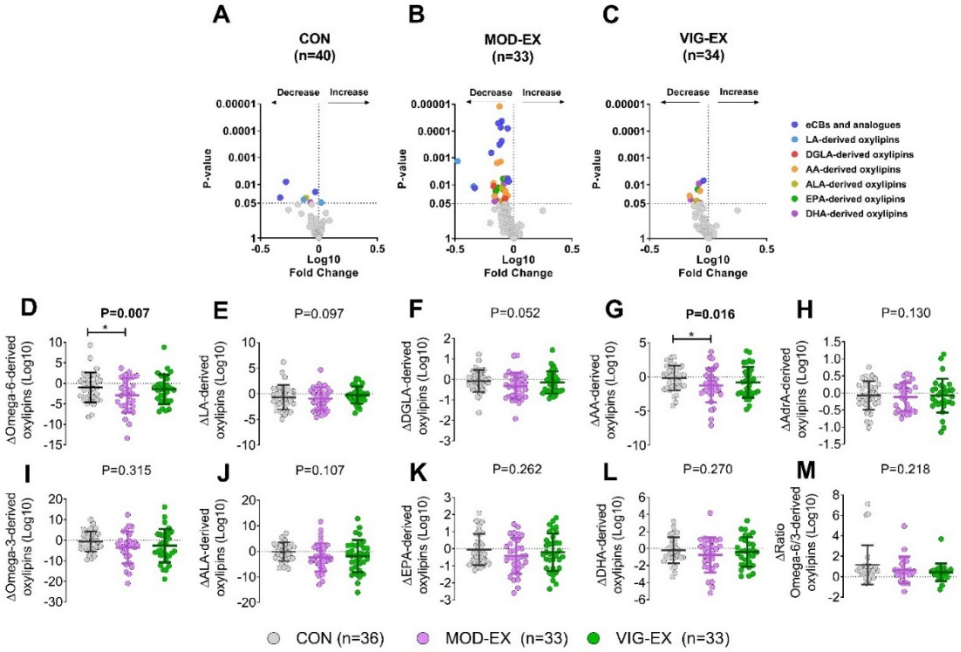

Figure 3. Long-term moderate, but not vigorous, intensity exercise training decreases plasma levels of omega-6-derived oxylipins in young adults.

A-C: Volcano plot showing the change of plasma levels of oxylipins, endocannabinoids, and their analogues after 24 weeks of intervention in control (A), moderate-intensity exercise (B), and vigorous-intensity exercise (C) groups. The X-axis shows the mean log10 fold change (post intervention minus baseline values) of the area peak ratio of each parameter, the Y-axis shows the P values obtained from paired t-test. Colored dots mean significantly increase/decrease after the intervention and grey dots were not significant after the intervention. D-N: For the analyses, the sum of omega-6 (D), LA-derived (E), DGLA-derived (F), AA-derived (G), AdrA-derived (H), omega-3 (I), ALA-derived (J), EPA-derived (L), DHA-derived (L), and the ratio of omega-6/omega-3 (M) 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) including the corresponding baseline values of each metabolite as a covariate. Data represent mean and standard deviation. * Symbol means significant differences between groups after post-hoc Bonferroni correction. Abbreviations: CON, control group; ECBs, endocannabinoids; MOD-EX, moderate-intensity exercise group; VIG-EX, vigorous-intensity exercise group; AA, arachidonic acid; AdrA, adrenic acid; ALA, α-linolenic acid; CON, control group; DGLA, dihomo-γ-linolenic acid; DHA, Docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MOD-EX, moderate-intensity exercise group; VIG-EX, vigorous-intensity exercise group.

One of the main degradation products of AEA and 2-AG is AA, levels of which decreased in response to MOD-EX. Besides, we found that MOD-EX significantly decreased AEA (-26.4%), DHEA (-21.8%), LEA (-22.5%), PEA (-10.4%), PDEA (-15.4%), POEA (-39.4%), and OEA (-20.9%) compared to the control group (all $P \leq 0.048$; **Figures 4A**, **E**, **G**, **I**, **J**, **K**, **L**, and **Table S4**). 2-AG (-23.7 %), POEA and OEA were the only metabolites of which plasma levels significantly decreased after MOD-EX compared to VIG-EX (all $P<0.05$; **Figures 4B**, **K**, **L**, and **Table S4**).

Overall, all significant results persisted after adjusting for both, baseline values and changes in PUFA intake (**Table S5**).

3.3 Exercise-induced acute changes in plasma levels of oxylipins and endocannabinoids are related to a better exercise capacity

120 min after acute endurance exercise, we found that changes in AdrA-, ALA- and EPA-derived oxylipins were positively correlated with the time to exhaustion (all $r \geq 0.539$, $P \leq 0.047$; **Figure S5C**). Similarly, changes in eCBs, and their analogues were positively correlated with VO_{2peak} relative to body weight (i.e., AEA, LEA, α-LEA, POEA, OEA; all $r \geq 0.554$, $P<0.05$; **Figure S5D**). 120 min after the bout of resistance exercise, the changes in 2-AG were positively correlated with adiposity ($r=0.786$, $P<0.001$; **Figure S5H**) and negatively correlated with handgrip strength as well as RMs leg press and bench press (all $r \leq -0.495$, $P \leq 0.043$; **Figure S5H**). However, changes in oxylipins, eCBs, and their analogues 3-min and 120-min after acute endurance and resistance exercise were not correlated with BFox and MFO (**Table S6**).

On the other hand, none of the changes observed on oxylipins, eCBs and their analogues induced by MOD-EX training were related to changes in adiposity, physical fitness capacity, cardiometabolic risk parameters, or dietary self-reported PUFA intake (data not shown). Changes induced by MOD-EX training in AA-derived oxylipins were negatively correlated with changes in MFO ($r=-0.520$; $P=0.009$; **Table S7).**

4. Discussion

Here, we provide an overview on acute and long-term effects of exercise on plasma levels of oxylipins, eCBs, and their analogues in young, sedentary adults. We found that endurance and resistance exercise acutely increase plasma levels of oxylipins derived from omega-6 (on average +50%) and omega-3 (+55%), as well as of eCBs and their analogues (+25%). In contrast, 24 weeks of MOD-EX training, but not VIG-EX training, reduced plasma levels of AA-derived omega-6 oxylipins (-12%), as well as eCBs and their analogues (-22%), although these decreases were not related to the observed reduction in adiposity or improvement in cardiorespiratory fitness. Our results suggest that these metabolites could be key mediators of the inflammatory and immune response to exercise.

The acute and long-term effects of exercise on oxylipins, eCBs, and their analogues observed in the present study are comparable to the effects on other inflammatory mediators. For instance, exercise is an acute pro-inflammatory stimulus that rapidly increases plasma levels of inflammatory molecules such as IL-6[41]. However, 120-180 minutes after exercise, the production of anti-inflammatory molecules, such as IL-10, dampening the pro-inflammatory response is increased, favoring the resolution of inflammation[41–43]. When exercise is repeated over time, such in a training program, there is a physiological adaptation leading to a reduced basal pro-inflammatory status (e.g., decrease of basal plasma levels of IL-6[44,45]. Therefore, exercise training might modulate plasma levels of oxylipins, eCBs and their analogues in a similar fashion to other inflammatory molecules (i.e., IL-6, IL-10).

4.1 Role of oxylipins, endocannabinoids, and their analogues in the acute response to exercise

We unveiled that both endurance and resistance exercises acutely increase the plasma levels of AA-, EPA-, and DHA-derived oxylipins in young sedentary adults. This is supported by previous studies that demonstrated that acute endurance exercise increases circulating levels of both omega-6 and omega-3 oxylipins (~2-8-fold)[26]. Exercise induces muscle damage accompanied by an increase of muscle inflammation and oxidative stress[7]. Immediately after exercise (3 min in our results), eicosanoids (i.e., prostaglandins, leukotrienes, and lipoxins), which are AA-derived oxylipins, are released to the systemic circulation as a pro-inflammatory response[7,8,10]. In this scenario, there is a rapid mobilisation of neutrophils,

which migrate from the blood stream to the damaged tissue[7], a process facilitated by AA-derived oxylipins, as we showed in our results, through the induction of vasodilation, vascular permeability and chemotaxis of neutrophils[7]. At a later stage during exercise recovery (120 min in our results), a set of anti-inflammatory and pro-resolution oxylipins such as EPA- and DHA-derived oxylipins, which are precursors of the E-series and D-series resolvins, protectins, and maresins, are released to the systemic circulation[7,8,10] as we observed in our results. These anti-inflammatory and pro-resolution lipid mediators cease the neutrophils infiltration, and recruit monocytes which eliminate neutrophils[7]. Therefore, omega-3 lipid mediators act as 'stop signals' to return to a non-inflammatory status after acute exercise and finally controlling the inflammatory and immune response to muscle damage[7,8,10]. Beyond the regulation of inflammation, these changes in plasma levels of oxylipins suggest a complex interplay between different tissues (i.e., skeletal muscle, adipose tissue), yet it remains unknown which tissues are the main contributors to the systemic levels of these metabolites[9,46].

Here, we demonstrate that eCBs and their analogues acutely increase after a maximal effort (endurance) test as well as after resistance exercise, suggesting that AEA levels can also be modified at high exercise intensities and by different types of exercise. On the other hand, previous studies have shown that the acute increase in circulating AEA after exercise is dependent on the intensity of exercise, increasing only after moderate-intensity exercise, but not vigorous-intensity exercise[47,48]. In fact, we observed that changes in eCBs and their analogues were positively correlated with VO_{2peak} relative to body weight, suggesting that the higher the increase of plasma levels of these molecules was after exercise, the higher was the cardiopulmonary capacity of the individual. Since cardiorespiratory fitness is a well-recognized marker of health status[49], these changes also reflect that a higher increase in eCBs and their analogues after exercise are linked to the health status of the individuals.

Akin to oxylipins, changes in eCBs and their analogues could be driven by an interplay between different cells (e.g., myocytes, neurons, adipocytes, or immune cells)[11,12]. Indeed, eCBs play a role in the modulation of inflammation and the immune system, through their binding to CB2R which is expressed in human leukocytes[50]. In this sense, eCBs exert both pro-inflammatory and anti-inflammatory or pro-resolution effects which might be crucial in the resolution of inflammation in response to exercise[50]. Another interesting role that has

been attributed to eCBs and their analogues is the modulation of the exercise analgesia effect or the so-called runner's high, but further studies are needed to investigate whether a causality exists underlying this relationship[12,47,51].

4.2 Role of oxylipins, endocannabinoids and their analogues in the long-term response to exercise training

High levels of omega-6 oxylipins and eCBs have been shown to be indicators of higher pro-inflammatory status, high adiposity levels, cardiovascular disruption, liver dysfunction, and/or oxidative stress[7,8,10,11,13,42,44,45], which concur with the findings observed in our cohort of young, sedentary adults[54]. Interestingly, we found that MOD-EX training, but not VIG-EX, was an effective therapy to decrease the plasma levels of these metabolites. Moderate-intensity exercise has demonstrated to induce higher analgesic effects than vigorous-intensity exercise through increased levels of eCBs levels[47,48]. Therefore, we are tempted to speculate that if acute MOD-EX is repeated over time, an adaptative response could arise, decreasing the basal levels of omega-6 oxylipins, eCBs, and their analogues. Our results also show that long-term changes in the plasma levels of these metabolites are not related to changes in body composition (i.e., adiposity and lean mass), physical fitness, cardiometabolic risk parameters or self-reported dietary PUFA intake. Interestingly, changes in AA-derived oxylipins were negatively correlated with changes in MFO. A decrease in pro-inflammatory oxylipins, such as AA-derived oxylipins, could induce alterations in genes involved in the regulation of fat oxidation in adipose, liver, and skeletal muscle tissues[55], and improve insulin sensitivity in these tissues[56], enhancing fat oxidation during exercise. However, the biological implications of the effect of exercise on the plasma levels of oxylipins, eCBs, and their analogues in humans require further investigation. Future studies are needed to elucidate whether a cross-talk between different tissues (e.g., skeletal muscle, adipose tissue, the gut) exists that might be responsible for the changes in the concentrations of these circulating metabolites. Furthermore, it is crucial to be aware that oxylipins and eCBs share common precursors (i.e., PUFAs) and metabolic enzymes (i.e., LOX and CYP)[9,53,57]. Intriguingly, we found that MOD-EX decreased AA-derived oxylipins that are also the end products of eCB catabolism. This finding suggests that AA precursors and derived products might play a crucial role during exercise training and should be further investigated[58].

5. Conclusions

This study showed that both endurance and resistance exercise acutely increase plasma levels of oxylipins, eCBs, and their analogues, whereas 24 weeks of moderate intensity exercise, but not vigorous exercise, decreases plasma levels of AA-derived omega-6 oxylipins, eCBs and their analogues in young sedentary adults. Our findings suggest that both acute and long-term effects of exercise on oxylipins and eCBs might be related to the inflammatory responses of exercise. Further studies are needed to understand the role of exercise in the modulation of the levels of these metabolites and the mechanisms behind exercise-benefits on inflammation.

Strengths and limitations

The current study shows a number of strengths and limitations. A major strength is the metabolomics-based methodology, targeting all omega-6 and omega-3 oxylipins, eCBs and their analogues. In addition, we have analyzed both the acute and long-term responses to exercise, and acute sessions of both endurance and resistance exercises. Nevertheless, a study limitation is the inclusion of only sedentary young adults, which does not allow extrapolation of the findings to older, children, unhealthy, or trained/active populations, and the relatively low sample size of males in the acute experiments. Due to the low volume of samples, we had to report area peak ratio as proxy of concentration of each metabolite following the Metabolomic Standard Initiative[59]. Moreover, the long-term effects of exercise were based on a concurrent intervention, without the isolation of long-term effects of aerobic and resistance training. Lastly, the sex heterogeneity of our cohort does not allow to know if the effects of exercise could be sex-dependent.

Funding

This study was supported by the Spanish Ministry of Economy and Competitiveness via the Fondo de Investigación Sanitaria del Instituto de Salud Carlos III (PI13/01393), and and PTA-12264, Retos de la Sociedad (DEP2016-79512-R) and the European Regional Development Fund (ERDF), the Spanish Ministry of Education (FPU16/02828, FPU17/01523, and FPU19/01609), the Fundación Iberoamericana de Nutrición (FINUT), the Redes Temáticas de Investigación Cooperativa RETIC (Red SAMID RD16/0022), the

AstraZeneca HealthCare Foundation, the University of Granada Plan Propio de Investigación 2016 -Excellence actions: Unit of Excellence on Exercise and Health (UCEES), the Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades (ERDF; ref. SOMM17/6107/UGR and DOC 01151), Junta de Andalucía, Consejería de Economía, Conocimiento, Empresas y Universidad (ref. P18-RT-4455), the Fundación Alfonso Martin Escudero, the Maria Zambrano fellowship by the Ministerio de Universidades y la Unión Europea–NextGenerationEU (RR_C_2021_04), the Netherlands CardioVascular Research Initiative: 'the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organisation for Health Research and Development and the Royal Netherlands Academy of Sciences' (CVON2017-20 GENIUS-2) to PCNR, and the Chinese Scholarship Council (CSC, No. 201707060012 for XD and No. 201707060012 for WY).

Acknowledgments

The authors would like to thank all the participants of this study for their time and effort.

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SUPPLEMENTAL MATERIALS

1. Acute exercise sessions

Endurance exercise trial

Participants walked at 3 km/h for 1 minute and at 4 km/h for 2 minutes for warming up (0% grade)[1]. Then, the test started by walking at 5.3 km/h and 0%. From that moment on, the treadmill inclination was increased by 1% every minute, until exhaustion was reached (1). At this point, participants started a 5-minute recovery walking at 4 km/h and 0% inclination (1). During the whole trial, participants were equipped with a heart rate monitor (Polar RS800CX, Polar Electro Öy, Kempele, Finland), 10 electrodes for electrocardiogram monitoring, and a Hans-Rudolph plastic mask (model 7400, Hans Rudolph Inc., Kansas City, MO, USA) connected to a preVent™ metabolic flow sensor (Medical graphics Corp, St Paul, MN, USA) for respiratory gas exchange analyses using a CPX Ultima CardioO2 gas exchange analysis system (Medical Graphics Corp, St Paul, MN, USA). During the test, respiratory gas exchange (oxygen consumption (VO2) and carbon dioxide production)) was recorded and the VO2peak was determined as the highest observed VO2 value, after excluding obvious artifacts if needed.

Resistance exercise trial

Participants first completed the maximum isometric strength test in leg press. After proper allocation in the leg press machine (A300 Leg Press, Model 2531, Keiser Corporation, Fresno CA, USA), participants performed two 3-second repetitions, 2 minutes apart, for which they were instructed and encouraged to push as hard as they could for the whole duration of the repetition.

Afterwards, participants performed the handgrip strength test by completing two repetitions with each hand, 1 minute apart using a Takei 5401 digital Grip-D hand dynamometer (Takei, Tokyo, Japan) (2). For the handgrip strength test, participants remained in a standing position, with the exercising arm parallel and slightly separated from the trunk. The participants were asked to squeeze the grip gradually and continuously, and as hard as possible. Men executed the test with the grip span of the dynamometer fixed at 5.5 cm, while it was adjusted to the individual's hand size for women, according to a validated equation[2]. The highest strength recorded in each hand was selected and the average between both hands was used for the analyses.

Then, participants performed the leg press 1-RM test in the above-mentioned leg press machine. After performing 1 set of 10 repetitions with a self-selected light weight for warming-up, they were instructed to perform 1 set of 8 repetitions selecting the resistance with which they could perform 15 repetitions as much. Later, after a 1-minute recovery, the resistance load was increased by the study personnel, aiming to set a load with which the participant could perform <10 repetitions, and participants were instructed to do as many repetitions as possible. The participants were instructed to stop exercising after 3-4 repetitions if they felt they could perform more than 10 repetitions with the resistance load. If they performed more than 10 repetitions, they rested for 5 minutes and repeated the test with a higher load. The maximum number of attempts for assessing the RM (in a set of <10 repetitions) was 3. Lastly, participants performed the bench press 1-RM test following the procedure described for the leg press, in a bench within a pneumatic power rack (Power rack, Model 3111, Keiser Corporation, Fresno CA, USA).The 1-RM of both exercises was estimated by the equation previously proposed (3).

2. Determination of plasma levels of oxylipins and endocannabinoid

2.1. Sample preparation

Oxylipins were extracted using liquid-liquid extraction under ice-cool conditions as previously described[4]. 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 (Table S2). 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, 4ºC) prior to collection of 40 µL of the supernatant, which was injected into the LC-MS/MS instrument.

2.2. Profiling of oxylipins and endocannabinoids using liquid chromatography – tandem mass spectrometry

Oxylipins and endocannabinoids analysis were performed using a previously validated method [4]. Briefly, extracted samples were analysed using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) connected to a SCIEX QTRAP 6500+ mass spectrometer (AB Sciex, Framingham, MA, USA). 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 0.7mL/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.

2.3. 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 monitored 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)[5]. Relative standard deviations (RSDs) of peak area ratios were calculated for each targeted analyte detected in the QC samples.

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Table S1. List of oxylipins, endocannabinoids, and endocannabinoids analogues measured.

Group	Abbreviation	Name (International Union of Pure and Applied Chemistry, IUPAC)	ChEBI ID	RSD in QC acute samples	RSD in QC long-term samples
	Omega-6 oxylipins				
LA-derived oxylipins	LA	9Z,12Z-octadecadienoic acid	17351	18.00	10.16
	9-HODE	(±)-9-hydroxy-10E,12Z-octadecadienoic acid	72651	4.55	7.61
	13-HODE	(±)-13-hydroxy-9Z,11E-octadecadienoic acid	72639	4.12	7.11
	9,10,13-TriHOME	9S,10S,13S-trihydroxy-11E-octadecenoic acid	34499	15.90	15.70
	9,12,13-TriHOME	9S,12S,13S-trihydroxy-10E-octadecenoic acid	34506	17.30	6.93
	9,10-EpOME	9,10-epoxy-12Z-octadecenoic acid	34494	11.38	7.80
	9,10-DiHOME	9,10-dihydroxy-12Z-octadecenoic acid	72663	ND	7.33
	12,13-EpOME	(±)-12(13)-epoxy-9Z-octadecenoic acid	38229	5.90	9.56
	12,13-DiHOME	12,13-dihydroxy-9Z-octadecenoic acid	72665	4.78	6.74
	10-NO2-LA	10-nitro,9Z,12Z-octadecadienoic acid	34125	16.16	13.26
	GLA	6Z,9Z,12Z-octadecatrienoic acid	28661	18.18	ND
DGLA-derived oxylipins	DGLA	8Z,11Z,14Z-eicosatrienoic acid	53486	13.82	23.92
	5-HETrE	5S-hydroxy-6E,8Z,11Z-eicosatrienoic acid	88359	18.06	ND
	8-HETrE	8S-hydroxy-9E,11Z,14Z-eicosatrienoic acid	140473	16.32	22.77
	15-HETrE	15S-hydroxy-8Z,11Z,13E-eicosatrienoic acid	88348	10.36	13.55
AA-derived oxylipins	AA	5Z,8Z,11Z,14Z-eicosatetraenoic acid	15843	6.10	13.45
	PGE2	9-oxo-11R,15S-dihydroxy-5Z,13E-prostadienoic acid	15551	5.93	35.97
	PGF2α	9α,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid	15553	3.20	ND
	2,3-dinor-8-iso-PGF2α	9α,11α,15S-trihydroxy-2,3-dinor-(8β)-prosta-5Z,13E-dien-1-oic acid	NA	15.33	ND
	13,14-dihydro-15-keto-PGF2α	9α,11α-dihydroxy-15-oxo-prost-5Z-en-1-oic acid	63976	5.90	ND
	TxB2	9S,11,15S-trihydroxy-thromboxa-5Z,13E-dien-1-oic acid	28728	14.33	6.95
	12-HHTrE	12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid	63977	16.78	8.11
	8,12-IPF2α-IV	(12α)-5,9α,11α-trihydroxy-prosta-6E,14Z-dien-1-oic acid	NA	3.17	7.20
	5-HETE	5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid	28209	6.37	10.43
	8-HETE	8-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid	34486	7.08	ND
	9-HETE	9-hydroxy-5Z,7E,11Z,14Z-eicosatetraenoic acid	72786	28.39	ND
	11-HETE	11-hydroxy-5Z,8Z,12E,14Z-eicosatetraenoic acid	72606	29.58	10.74
	12-HETE	12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid	19138	14.27	11.41
	15-HETE	15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid	64017	6.99	9.33
	20-HETE	20-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	34306	6.94	9.35
	5,6-DiHETrE	5,6-dihydroxy-8Z,11Z,14Z-eicosatrienoic acid	63974	5.76	9.02
	8,9-DiHETrE	8,9-dihydroxy-5Z,11Z,14Z-eicosatrienoic acid	63970	7.56	9.91
	11,12-EpETrE	11,12-epoxy-5Z,8Z,14Z-eicosatrienoic acid	34130	16.16	ND
	11,12-DiHETrE	11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid	63969	5.28	8.44
	14,15-EpETrE	14,15-epoxy-5Z,8Z,11Z-eicosatrienoic acid	34157	13.81	19.47
	14,15-DiHETrE	14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid	63966	4.48	7.1
AdrA-derived oxylipins	AdrA	7Z,10Z,13Z,16Z-docosatetraenoic acid	53487	16.2	22.09
	1a,1b-dihomo-PGF2α	1a,1b-dihomo-9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid	NA	7.10	22.14
	Omega-3 oxylipins				
LA-derived oxylipins	ALA	9Z,12Z,15Z-octadecatrienoic acid	27432	10.78	9.84
	9-HOTrE	9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid	80447	5.39	7.61
	12,13-DiHODE	(±)-12,13-dihydroxy-9Z,15Z-octadecadienoic acid	88461	4.24	5.80

		Continued			
EPA-derived oxylipins	EPA	5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid	28364	14.56	8.55
	5-HEPE	(±)-5-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid	72801	9.58	13.12
	12-HEPE	(±)-12-hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid	72645	20.29	12.07
	15-HEPE	(±)-15-hydroxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid	72627	12.95	ND
	14,15-DiHETE	(±)-14,15-dihydroxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid	88459	5.20	8.02
	17,18-DiHETE	(±)-17,18-dihydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	88349	5.25	9.06
	DPA	7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid	61204	9.68	13.97
DHA-derived oxylipins	DHA	4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid	28125	6.11	9.80
	4-HDoHE	(±)-4-hydroxy-5E,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid	72624	18.37	14.25
	7-HDoHE	(+/-)-7-hydroxy-4Z,8E,10Z,13Z,16Z,19Z-docosahexaenoic acid	72623	29.33	ND
	8-HDoHE	(±)-8-hydroxy-4Z,6E,10Z,13Z,16Z,19Z-docosahexaenoic acid	72610	ND	19.71
	10-HDoHE	(+/-)-10-hydroxy-4Z,7Z,11E,13Z,16Z,19Z-docosahexaenoic acid	72640	27.78	ND
	11-HDoHE	(±)-11-hydroxy-4Z,7Z,9E,13Z,16Z,19Z-docosahexaenoic acid	72794	24.17	17.09
	13-HDoHE	(±)-13-hydroxy-4Z,7Z,10Z,14E,16Z,19Z-docosahexaenoic acid	72608	23.01	12.28
	14-HDoHE	(±)-14-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid	72647	9.46	14.75
	16-HDoHE	(±)-16-hydroxy-4Z,7Z,10Z,13Z,17E,19Z-docosahexaenoic acid	72613	14.44	15.42
	17-HDoHE	(±)-17-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid	72637	17.922	9.05
	20-HDoHE	(±)-20-hydroxy-4Z,7Z,10Z,13Z,16Z,18E-docosahexaenoic acid	72615	20.23	23.60
	19,20-EpDPE	(±)-19(20)-epoxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid	72653	8.17	13.45
	19,20-DiHDPA	(±)-19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid	72657	5.07	7.89
	Endocannabinoids and their analogues				
	AEA	N-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-ethanolamine	2700	8.61	11.33
	2-AG	2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycerol	52392	12.97	16.80
	2-LG	2-(9Z,12Z-Octadecadienoyl)-glycerol	NA	ND	19.17
	2-OG	2-(9Z-octadecenoyl)-sn-glycerol	73990	ND	27.71
	DHEA	N-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-ethanolamine	85252	9.41	16.53
	DGLEA	N-(8Z,11Z,14Z-eicosatrienoyl)-ethanolamine	34488	13.08	26.65
	LEA	N-(9Z,12Z-octadecadienoyl)-ethanolamine	64032	4.20	5.3
	α-LEA	N-(9Z,12Z,15Z-octadecatrienoyl)-ethanolamine	89605	9.4	14.9
	PEA	N-hexadecanoyl-ethanolamine	71464	5.47	4.05
	PDEA	N-(Pentadecanoyl)-ethanolamine	N/A	21.47	30.3
	POEA	N-(9Z-hexadecenoyl)-ethanolamine	71465	9.38	13.5
	OEA	N-(9Z-octadecenoyl)-ethanolamine	71466	19.00	7.70
	SEA	N-(Octadecanoyl)-ethanolamine	85299	8.25	10.62

ChEBI, Chemical Entities of Biological Interest; N/A, not available; ND, not detected; QC, quality control; RSD, relative standard error.

Table S2. List of internal standards used in the LC/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-iPF2a-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-PGF2 α	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-PGF2 α	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-iPF2 α -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-iPF2 α -VI	(12α)-5,9α,11α-trihydroxy-prosta-6*E*,14*Z*-dien-1-oic acid-d11
d17-10-Nitrooleate	10-nitro,9Z,12Z-octadecadienoic acid-d17
d11-14,15-DiHETrE	14,15-dihydroxy-5*Z*,8*Z*,11*Z*-eicosatrienoic acid-d11
d4-9(S)-HODE	9*S*-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(S)-HETE	12*S*-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid-d8
d8-5(S)-HETE	5*S*-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. Baseline levels of oxylipins, endocannabinoids, and endocannabinoids analogues measured.

		Acute effect of exercise				Long-term effect of exercise					
		Endurance (n=14)		Resistance (n=17)		CON (n=36)		MOD-EX (n=33)		VIG-EX (n=33)	
Group	Oxylipin	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Omega-6 oxylipins											
LA-derived oxylipins	LA	-3.75	1.01	-3.46	0.76	1.01	0.07	1.00	0.09	1.00	0.11
	9-HODE	0.70	0.65	0.69	0.68	0.67	0.20	0.64	0.20	0.61	0.16
	13-HODE	0.46	0.62	0.24	0.61	-0.02	0.19	-0.05	0.19	-0.10	0.16
	9,10,13-TriHOME	1.24	0.60	0.81	0.64	0.26	0.59	0.26	0.51	0.10	0.57
	9,12,13-TriHOME	0.97	1.63	0.18	1.68	-0.13	0.51	-0.13	0.56	-0.26	0.48
	9,10-EpOME	-4.62	1.25	-4.75	1.30	-1.31	0.24	-1.40	0.15	-1.44	0.20
	9,10-DiHOME	-	-	-	-	0.43	0.27	0.26	0.27	0.24	0.27
	12,13-EpOME	-2.59	1.11	-3.09	0.89	-0.75	0.22	-0.85	0.20	-0.89	0.26
	12,13-DiHOME	0.38	0.77	0.13	0.86	-0.07	0.18	-0.12	0.19	-0.14	0.18
	10-NO2-LA	-4.38	0.60	-4.53	1.18	-1.22	0.26	-1.35	0.31	-1.32	0.30
	GLA	3.13	1.25	3.70	1.16	-	-	-	-	-	-
DGLA-derived oxylipins	DGLA	-3.83	0.59	-3.33	0.92	-0.06	0.23	-0.08	0.23	-0.07	0.22
	5-HETrE	-5.21	0.29	-5.21	0.54	-	-	-	-	-	-
	8-HETrE	-2.93	0.44	-2.92	0.81	-1.15	0.26	-1.10	0.29	-1.09	0.24
	15-HETrE	-5.89	0.57	-5.68	0.81	-1.82	0.18	-1.87	0.17	-1.85	0.15
AA-derived oxylipins	AA	1.36	0.45	1.72	0.51	1.78	0.12	1.78	0.15	1.82	0.16
	PGE2	-4.15	1.14	-4.14	1.38	-1.24	0.32	-1.30	0.35	-1.20	0.31
	PGF2α	-5.75	0.08	-6.01	1.15	-	-	-	-	-	-
	2,3-dinor-8-iso-PGF2α	-7.74	0.72	-7.72	0.83	-	-	-	-	-	-
	13,14-dihydro-15-keto-PGF2α	-9.53	0.66	-9.28	0.92	-	-	-	-	-	-
	TxB2	-1.18	2.23	-1.03	1.62	-0.56	0.42	-0.69	0.57	-0.54	0.42
	12-HHTrE	-2.28	1.30	-2.70	1.61	-0.86	0.35	-0.96	0.48	-0.83	0.39
	8,12-IPF2α-IV	-5.91	0.34	-5.97	0.33	-1.66	0.10	-1.66	0.11	-1.66	0.11
	5-HETE	-4.57	0.59	-4.09	0.76	-1.25	0.21	-1.19	0.21	-1.19	0.20
	8-HETE	-4.83	0.28	-4.63	0.51	-	-	-	-	-	-
	9-HETE	-1.51	0.79	-1.56	1.33	-	-	-	-	-	-
	11-HETE	-1.49	0.82	-1.58	1.33	-0.67	0.15	-0.67	0.16	-0.64	0.15
	12-HETE	-1.52	0.98	-1.49	1.07	-0.70	0.27	-0.72	0.25	-0.68	0.31
	15-HETE	-4.18	0.48	-3.98	0.60	-1.19	0.13	-1.20	0.13	-1.19	0.14
	20-HETE	-4.22	0.54	-4.09	0.65	-0.97	0.13	-0.97	0.18	-0.99	0.17
	5,6-DiHETrE	-8.15	0.46	-7.80	0.41	-2.30	0.15	-2.25	0.21	-2.29	0.18
	8,9-DiHETrE	-8.90	0.34	-8.69	0.38	-2.40	0.13	-2.38	0.17	-2.37	0.13
	11,12-EpETrE	-11.80	0.48	-11.45	0.62	-	-	-	-	-	-
	11,12-DiHETrE	-6.22	0.24	-6.05	0.51	-1.54	0.08	-1.55	0.14	-1.55	0.11
	14,15-EpETrE	-11.33	0.67	-10.93	0.71	-2.99	0.14	-3.01	0.19	-3.00	0.20
	14,15-DiHETrE	-5.12	0.26	-4.97	0.41	-1.34	0.07	-1.35	0.12	-1.35	0.11
AdrA - deriv	AdrA	-3.24	1.02	-3.02	1.09	-0.01	0.24	-0.03	0.28	-0.03	0.21
	1a,1b-dihomo-PGF2α	-8.12	0.45	-8.40	0.81	-2.09	0.24	-2.20	0.22	-2.18	0.29

Omega-3 oxylipins											
LA-derived oxylipins	ALA	3.72	1.23	4.24	0.86	1.01	0.20	1.03	0.19	1.04	0.19
	9-HOTrE	-4.68	0.78	-4.52	1.02	-1.49	0.32	-1.51	0.21	-1.53	0.21
	12,13-DiHODE	-1.61	0.62	-1.65	1.00	-0.52	0.28	-0.61	0.16	-0.57	0.23
EPA-derived oxylipins	EPA	-0.97	0.63	-0.63	1.03	1.21	0.23	1.26	0.24	1.30	0.23
	5-HEPE	-7.77	0.82	-7.46	1.18	-2.09	0.26	-2.01	0.24	-2.00	0.25
	12-HEPE	-6.11	1.22	-6.12	1.64	-1.57	0.39	-1.64	0.39	-1.57	0.46
	15-HEPE	-8.45	0.87	-8.08	0.87	-	-	-	-	-	-
	14,15-DiHETE	-9.65	0.47	-9.72	0.63	-2.76	0.19	-2.74	0.20	-2.69	0.17
	17,18-DiHETE	-7.31	0.42	-7.55	0.77	-2.02	0.20	-2.01	0.22	-1.97	0.17
	DPA	-9.00	0.85	-8.64	0.90	-1.08	0.19	-1.04	0.19	-0.95	0.19
DHA-derived oxylipins	DHA	1.63	0.62	1.75	0.86	2.09	0.16	2.12	0.16	2.19	0.16
	4-HDoHE	-5.73	1.18	-5.92	1.43	-1.82	0.22	-1.78	0.29	-1.77	0.24
	7-HDoHE	-7.16	0.52	-6.83	1.46	-	-	-	-	-	-
	8-HDoHE	-	-	-	-	-1.78	0.21	-1.73	0.19	-1.68	0.23
	10-HDoHE	-6.03	0.60	-5.61	1.18	-	-	-	-	-	-
	11-HDoHE	-5.37	1.24	-5.17	1.59	-1.23	0.37	-1.25	0.36	-1.17	0.40
	13-HDoHE	-5.69	0.89	-5.51	1.57	-1.76	0.24	-1.81	0.22	-1.70	0.24
	14-HDoHE	-5.01	1.18	-4.78	1.29	-1.40	0.40	-1.45	0.46	-1.40	0.43
	16-HDoHE	-4.20	0.59	-4.04	1.05	-1.57	0.21	-1.57	0.15	-1.51	0.19
	17-HDoHE	-4.40	0.48	-4.35	1.04	-1.45	0.20	-1.46	0.16	-1.42	0.21
	20-HDoHE	-4.47	0.64	-4.10	1.49	-1.43	0.35	-1.43	0.36	-1.34	0.42
	19,20-EpDPE	-9.91	1.11	-9.88	1.05	-2.68	0.22	-2.64	0.18	-2.60	0.18
	19,20-DiHDPA	-5.30	0.53	-5.47	0.76	-1.53	0.17	-1.51	0.17	-1.49	0.15
Endocannabinoids and their analogues											
	AEA	-3.17	0.38	-2.85	0.46	-0.91	0.13	-0.88	0.14	-0.91	0.16
	2-AG	0.23	0.53	0.27	0.76	-2.18	0.61	-2.17	0.48	-2.16	0.43
	2-LG	-	-	-	-	-1.90	0.88	-2.08	0.77	-2.06	0.54
	2-OG	-	-	-	-	-3.17	0.89	-3.21	0.80	-3.34	0.59
	DHEA	-5.02	0.53	-4.78	0.61	-1.16	0.30	-1.20	0.13	-1.18	0.12
	DGLEA	-6.81	0.62	-6.54	0.65	-1.68	0.20	-1.65	0.16	-1.70	0.18
	LEA	-2.67	0.41	-2.32	0.41	-0.69	0.15	-0.70	0.15	-0.75	0.14
	α-LEA	-7.36	0.36	-7.09	0.37	-2.18	0.15	-2.20	0.14	-2.18	0.16
	PEA	-0.91	0.29	-0.55	0.36	0.23	0.06	0.23	0.06	0.22	0.05
	PDEA	-6.19	0.39	-6.16	0.65	-1.65	0.12	-1.65	0.15	-1.64	0.13
	POEA	-2.82	0.72	-2.37	1.07	-0.72	0.33	-0.66	0.29	-0.68	0.19
	OEA	-1.11	0.30	-0.74	0.42	-0.18	0.11	-0.18	0.13	-0.20	0.11
	SEA	-0.20	0.28	0.14	0.42	0.10	0.07	0.08	0.07	0.10	0.07

Data are represented as mean and standard deviation (SD) of the log2 area peak ratio of each parameter. ***Abbreviations***: CON, control group: MOD-EX, moderate-intensity exercise group; VIG-EX, vigorous-intensity exercise group..

Table S4. Changes in oxylipins, endocannabinoids and their analogues after the exercise intervention.

	CON (n=36)			MOD-EX (n=33)			VIG-EX (n=33)				
	Mean	SE	95% CI	Mean	SE	95% CI	Mean	SE	95% CI	η^2	P value
Oxylipins											
ΔOmega-6-derived oxylipins (log10)	-0.718	0.499	[-0.171; 0.274]	-3.088	0.541	[-4.164; -2.013]	-1.590	0.541	[-2.665; -0.515]	0.110	**0.007**
ΔLA-derived oxylipins (log10)	-0.158	0.276	[-0.706; 0.391]	-1.012	0.280	[-1.567; -0.457]	-0.699	0.284	[-1.263; -0.135]	0.050	0.097
ΔDGLA-derived oxylipins (log10)	-0.087	0.074	[-0.234; -0.061]	-0.334	0.077	[-0.488; -0.180]	-0.122	0.078	[-0.276; 0.031]	0.058	0.052
ΔAA-derived oxylipins (log10)	-0.189	0.279	[-0.742; 0.365]	-1.388	0.301	[-1.986; -0.791]	-0.604	0.297	[-1.193; -0.016]	0.084	**0.016**
ΔAdrA-derived oxylipins (log10)	0.002	0.058	[-0.114; 0.117]	-0.168	0.061	[-0.288; -0.048]	-0.109	0.060	[-0.229; 0.011]	0.045	0.130
ΔOmega-3-derived oxylipins (log10)	-1.146	0.993	[-3.121; 0.829]	-3.367	1.062	[-5.480; -1.255]	-2.327	1.008	[-4.332; -0.322]	0.027	0.315
ΔALA-derived oxylipins (log10)	-0.347	0.676	[-1.689; 0.994]	-2.384	0.696	[-3.765; -1.003]	-1.679	0.696	[-3.061; -0.298]	0.045	0.107
ΔEPA-derived oxylipins (log10)	-0.101	0.154	[-0.406; 0.204]	-0.434	0.163	[-0.757; -0.111]	-0.119	0.161	[-0.439; 0.200]	0.027	0.262
ΔDHA-derived oxylipins (log10)	-0.272	0.278	[-0.825; 0.280]	-0.839	0.297	[-1.429; -0.249]	-0.238	0.288	[-0.810; 0.335]	0.030	0.270
ΔRatio omega-6/3-derived oxylipins (log10)	1.134	0.277	[0.581; 1.687]	0.667	0.305	[0.058; 1.276]	0.437	0.293	[-0.147; 1.021]	0.041	0.218
Endocannabinoids and their analogues											
ΔAEA (log10)	-0.005	0.022	[-0.048; 0.039]	-0.121	0.023	[-0.167; -0.076]	-0.053	0.023	[-0.099; -0.008]	0.122	**0.002**
Δ2-AG (log10)	-0.323	0.065	[-0.453; -0.193]	-0.309	0.070	[-0.449; -0.170]	-0.055	0.069	[-0.192; 0.083]	0.093	**0.010**
Δ2-LG (log10)	-0.255	0.096	[-0.445; -0.064]	-0.140	0.100	[-0.339; 0.058]	0.016	0.100	[-0.183; 0.214]	0.037	0.154
Δ2-OG (log10)	-0.226	0.091	[-0.407; -0.044]	-0.158	0.095	[-0.347; 0.032]	0.173	0.097	[-0.19; 0.366]	0.093	**0.009**
ΔDHEA (log10)	-0.029	0.021	[-0.071; 0.013]	-0.111	0.022	[-0.154; -0.067]	-0.038	0.022	[-0.081; 0.006]	0.082	**0.016**
ΔDGLEA (log10)	-0.028	0.027	[-0.082; 0.025]	-0.101	0.029	[-0.158; -0.043]	-0.031	0.029	[-0.088; 0.025]	0.041	0.137
ΔLEA (log10)	0.007	0.017	[-0.027; 0.041]	-0.098	0.018	[-0.134; -0.063]	-0.051	0.018	[-0.086; -0.015]	0.157	**<0.001**
Δα-LEA (log10)	-0.010	0.024	[-0.058; 0.039]	-0.060	0.025	[-0.110; -0.009]	-0.055	0.025	[-0.106; -0.005]	0.025	0.292
ΔPEA (log10)	-0.011	0.009	[-0.028; 0.007]	-0.044	0.009	[-0.062; -0.026]	-0.015	0.009	[-0.033; 0.003]	0.077	**0.021**
ΔPDEA (log10)	0.013	0.019	[-0.026; 0.051]	-0.056	0.020	[-0.096; -0.016]	-0.030	0.020	[-0.070; 0.010]	0.060	**0.048**
ΔPOEA (log10)	0.031	0.036	[-0.040; 0.103]	-0.173	0.038	[-0.248; -0.098]	-0.014	0.038	[-0.089; 0.061]	0.144	**<0.001**
ΔOEA (log10)	0.008	0.017	[-0.026; 0.042]	-0.097	0.018	[-0.133; -0.061]	-0.007	0.018	[-0.043; 0.029]	0.171	**<0.001**
ΔSEA (log10)	-0.031	0.011	[-0.052; -0.010]	-0.053	0.011	[-0.075; -0.031]	-0.034	0.011	[-0.056; -0.011]	0.024	0.310

Data is presented as estimated marginal means, standard error (SE) and 95% confidence interval (CI). Depicted η^2 and P values are obtained from obtained from analyses of covariance (ANCOVA) including the corresponding baseline values of each metabolite as a covariate. ***Abbreviations***: 2-AG, 2-arachidonylglycerol; AA, arachidonic acid; AdrA, adrenic acid; AEA, anandamide; ALA, α-linolenic acid; CON, control group; DGLA, dihomo-γ-linolenic acid; DGLEA, dihomo-gamma-linolenoyl ethanolamide; DHA, Docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamide; EPA, eicosapentaenoic acid; LA, linoleic acid; LEA, linoleoyl ethanolamide; MOD-EX, moderate-intensity exercise group; OEA, oleoyl ethanolamine; PDEA, pentadecanoyl ethanolamide; PEA, palmitoyl ethanolamide; POEA, palmitoleoyl ethanolamide; SEA, stearoyl Ethanolamide; VIG-EX, vigorous-intensity exercise group; α-LEA, α-Linolenoyl ethanolamide.

Table S5. Changes in oxylipins and endocannabinoids adjusting for baseline values and changes in PUFA intake.

	P values
ΔOmega-6-derived oxylipins (log10)	**0.006**
ΔLA-derived oxylipins (log10)	0.081
ΔDGLA-derived oxylipins (log10)	**0.047**
ΔAA-derived oxylipins (log10)	**0.021**
ΔAdrA-derived oxylipins (log10)	0.110
ΔOmega-3-derived oxylipins (log10)	0.285
ΔALA-derived oxylipins (log10)	0.131
ΔEPA-derived oxylipins (log10)	0.270
ΔDHA-derived oxylipins (log10)	0.241
ΔRatio omega-6/3-derived oxylipins (log10)	0.122
ΔAEA (log10)	**0.002**
Δ2-AG (log10)	**0.008**
Δ2-LG (log10)	0.185
Δ2-OG (log10)	**0.011**
ΔDHEA (log10)	**0.018**
ΔDGLEA (log10)	0.165
Δα-LEA (log10)	0.362
ΔPEA (log10)	**0.024**
ΔPDEA (log10)	0.074
ΔPOEA (log10)	**0.001**
ΔOEA (log10)	**<0.001**
ΔSEA (log10)	0.197

P value obtained from analyses of covariance (ANCOVA) adjusting for baseline values and changes in PUFA intake. ***Abbreviations***: 2-AG, 2-arachidonylglycerol; AA, arachidonic acid; AdrA, adrenic acid; AEA, anandamide; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DGLEA, dihomo-gamma-linolenoyl ethanolamide; DHA, Docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamide; EPA, eicosapentaenoic acid; LA, linoleic acid; LEA, linoleoyl ethanolamide; OEA, oleoyl ethanolamine; PDEA, pentadecanoyl ethanolamide; PEA, palmitoyl ethanolamide; POEA, palmitoleoyl ethanolamide; SEA, stearoyl Ethanolamide; α-LEA, α-Linolenoyl ethanolamide.

Table S6. Pearson correlation of 3min and 120min fold-change relative to baseline of oxylipins and endocannabinoids with basal fat oxidation and maximal fat oxidation**.**

	Endurance				Resistance	
	BFox		MFO		BFox	
	r	P	r	P	r	P
3-min fold change relative to baseline						
Oxylipins						
Omega-6	0.080	0.064	0.761	0.310	0.010	0.974
LA	0.220	0.450	0.185	0.565	0.250	0.389
DGLA	0.346	0.225	-0.14	0.589	0.134	0.648
AA	-0.104	0.735	0.285	0.396	-0.069	0.814
AdrA	-0.229	0.431	0.363	0.246	-0.077	0.793
Omega-3	0.370	0.236	-0.179	0.620	0.402	0.1958
ALA	0.056	0.848	-0.186	0.563	0.150	0.608
EPA	0.246	0.397	-0.001	0.998	0.332	0.246
DHA	0.381	0.222	-0.304	0.392	0.411	0.184
Ratio omega 6/3	0.442	0.150	-0.094	0.797	0.536	0.073
Endocannabinoids						
AEA	0.325	0.256	-0.056	0.863	0.165	0.574
2-AG	-0.050	0.865	0.081	0.801	0.279	0.334
DHEA	0.157	0.592	0.122	0.705	0.088	0.765
DGLEA	0.050	0.865	0.333	0.291	-0.443	0.113
LEA	0.163	0.577	-0.252	0.429	0.179	0.506
α-LEA	-0.252	0.385	0.270	0.395	0.102	0.728
PEA	0.054	0.854	-0.250	0.433	0.392	0.133
PDEA	-0.113	0.700	-0.100	0.758	-0.078	0.790
POEA	0.091	0.758	-0.068	0.834	0.221	0.411
OEA	0.377	-0.089	-0.106	0.742	0.038	0.896
SEA	-0.089	0.762	-0.237	0.459	-0.312	0.240
120-min fold change relative to baseline						
Oxylipins						
Omega-6	0.064	0.828	0.320	0.310	0.170	0.561
LA	0.165	0.574	0.254	0.426	0.463	0.096
DGLA	0.344	0.229	0.042	0.989	-0.147	0.615
AA	-0.018	0.955	0.607	0.050	0.255	0.378
AdrA	-0.116	0.693	0.334	0.289	-0.077	0.793
Omega-3	0.298	0.347	-0.246	0.494	0.402	0.195
ALA	-0.211	0.469	0.088	0.787	-0.050	0.866
EPA	-0.070	0.812	0.252	0.430	0.332	0.246
DHA	0.442	0.150	-0.237	0.510	0.531	0.076
Ratio omega 6/3	-0.080	0.804	-0.144	0.692	0.161	0.618
Endocannabinoids						
AEA	-0.138	0.638	0.350	0.265	0.133	0.651
2-AG	-0.082	0.780	0.099	0.760	0.098	0.739
DHEA	-0.006	0.985	0.142	0.659	0.087	0.767
DGLEA	-0.039	0.895	-0.089	0.782	-0.166	0.570
LEA	0.008	0.979	0.017	0.959	0.042	0.886
α-LEA	-0.076	0.797	0.334	0.288	-0.058	0.843
PEA	-0.309	0.282	0.189	0.557	0.668	**0.009**
PDEA	0.025	0.933	0.060	0.852	0.195	0.505
POEA	0.139	0.637	0.307	0.332	0.268	0.354
OEA	-0.002	0.994	0.053	0.869	0.258	0.372
SEA	-0.198	0.496	-0.037	0.909	0.263	0.364

P value and r obtained from Pearson correlation analyses. *Abbreviations*: 2-AG, 2-arachidonylglycerol; AA, arachidonic acid; AdrA, adrenic acid; AEA, anandamide; ALA, α-linolenic acid; BFox, basal fat oxidation test; DGLA, dihomo-γ-linolenic acid; DGLEA, dihomo-gamma-linolenoyl ethanolamide; DHA, Docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamide; EPA, eicosapentaenoic acid; LA, linoleic acid; LEA, linoleoyl ethanolamide; MFO, Maximal fat oxidation test, OEA, oleoyl ethanolamine; PDEA, pentadecanoyl ethanolamide; PEA, palmitoyl ethanolamide; POEA, palmitoleoyl ethanolamide; SEA, stearoyl Ethanolamide; α-LEA, α-Linolenoyl ethanolamide.

Table S7. Pearson correlation of changes in oxylipins and endocannabinoids with changes in basal fat oxidation and maximal fat oxidation after 24-week of exercise intervention**.**

	CON (n= 36)				**MOD-EX (n= 33)**				**VIG-EX (n= 33)**			
	ΔBFox		**ΔMFO**		**ΔBFox**		**ΔMFO**		**ΔBFox**		**ΔMFO**	
	r	P	r	P	r	P	r	P	r	P	r	P
Oxylipins												
ΔOmega-6	-0.021	0.929	0.275	0.193	-0.172	0.495	-0.141	0.532	-0.104	0.692	-0.306	0.203
ΔLA	-0.071	0.758	0.321	0.126	0.048	0.841	0.352	0.085	0.223	0.345	0.016	0.946
ΔDGLA	-0.117	0.604	0.178	0.394	0.015	0.950	-0.010	0.963	-0.172	0.455	-0.421	**0.045**
ΔAA	0.075	0.741	0.108	0.609	-0.280	0.261	-0.520	**0.009**	-0.049	0.837	-0.275	0.215
ΔAdrA	0.119	0.608	-0.188	0.378	0.082	0.730	-0.210	0.325	-0.542	**0.020**	-0.440	**0.046**
ΔOmega-3	0.059	0.806	-0.019	0.936	-0.303	0.254	-0.158	0.505	-0.124	0.602	-0.126	0.596
ΔALA	-0.179	0.427	-0.074	0.730	-0.111	0.641	-0.058	0.778	-0.119	0.608	-0.193	0.377
ΔEPA	0.427	**0.047**	0.126	0.549	-0.361	0.129	-0.077	0.716	0.085	0.715	0.033	0.882
ΔDHA	0.573	**0.008**	-0.120	0.605	-0.367	0.147	-0.340	0.131	-0.017	0.945	0.003	0.990
ΔRatio omega 6/3	-0.063	0.805	-0.163	0.506	-0.066	0.822	-0.088	0.738	-0.752	**0.001**	-0.752	**0.001**
Endocannabinoids												
ΔAEA	0.188	0.403	0.283	0.170	0.102	0.668	-0.045	0.826	-0.001	0.996	-0.275	0.203
Δ2-AG	-0.303	0.171	0.231	0.266	0.204	0.403	0.185	0.375	-0.373	0.096	-0.081	0.712
Δ2-LG	-0.256	0.251	-0.013	0.951	0.138	0.563	0.348	0.082	-0.328	0.147	-0.046	0.836
Δ2-OG	-0.211	0.346	0.057	0.787	0.187	0.429	0.189	0.356	-0.352	0.128	-0.071	0.749
ΔDHEA	0.397	0.075	0.271	0.200	-0.052	0.827	-0.065	0.754	0.150	0.516	-0.013	0.955
ΔDGLEA	-0.247	0.268	0.171	0.414	0.207	0.395	-0.107	0.612	0.005	0.984	-0.228	0.307
ΔLEA	-0.007	0.976	0.221	0.288	0.047	0.843	0.040	0.848	-0.081	0.728	-0.301	0.162
Δα-LEA	0.163	0.467	0.165	0.432	-0.271	0.248	0.001	0.995	0.030	0.897	0.018	0.934
ΔPEA	0.088	0.698	0.341	0.095	0.060	0.809	-0.125	0.553	0.176	0.446	-0.170	0.439
ΔPDEA	0.065	0.773	0.179	0.393	-0.526	**0.017**	0.025	0.904	0.092	0.691	0.128	0.562
ΔPOEA	-0.318	0.150	0.187	0.371	-0.144	0.544	-0.076	0.711	-0.055	0.811	-0.138	0.530
ΔOEA	0.105	0.642	0.146	0.487	-0.096	0.687	-0.105	0.608	0.055	0.812	-0.240	0.270
ΔSEA	-0.036	0.875	0.349	0.087	-0.219	0.353	-0.124	0.547	-0.164	0.477	-0.124	0.572

P value and r obtained from Pearson correlation analyses. *Abbreviations*: 2-AG, 2-arachidonylglycerol; AA, arachidonic acid; AdrA, adrenic acid; AEA, anandamide; ALA, α-linolenic acid; BFox, basal fat oxidation test, CON, control group; DGLA, dihomo-γ-linolenic acid; DGLEA, dihomo-gamma-linolenoyl ethanolamide; DHA, Docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamide; EPA, eicosapentaenoic acid; LA, linoleic acid; LEA, linoleoyl ethanolamide; MFO, Maximal fat oxidation; MOD-EX, moderate-intensity exercise group; OEA, oleoyl ethanolamine; PDEA, pentadecanoyl ethanolamide; PEA, palmitoyl ethanolamide; POEA, palmitoleoyl ethanolamide; SEA, stearoyl Ethanolamide; VIG-EX, vigorous-intensity exercise group; α-LEA, α-Linolenoyl ethanolamide.

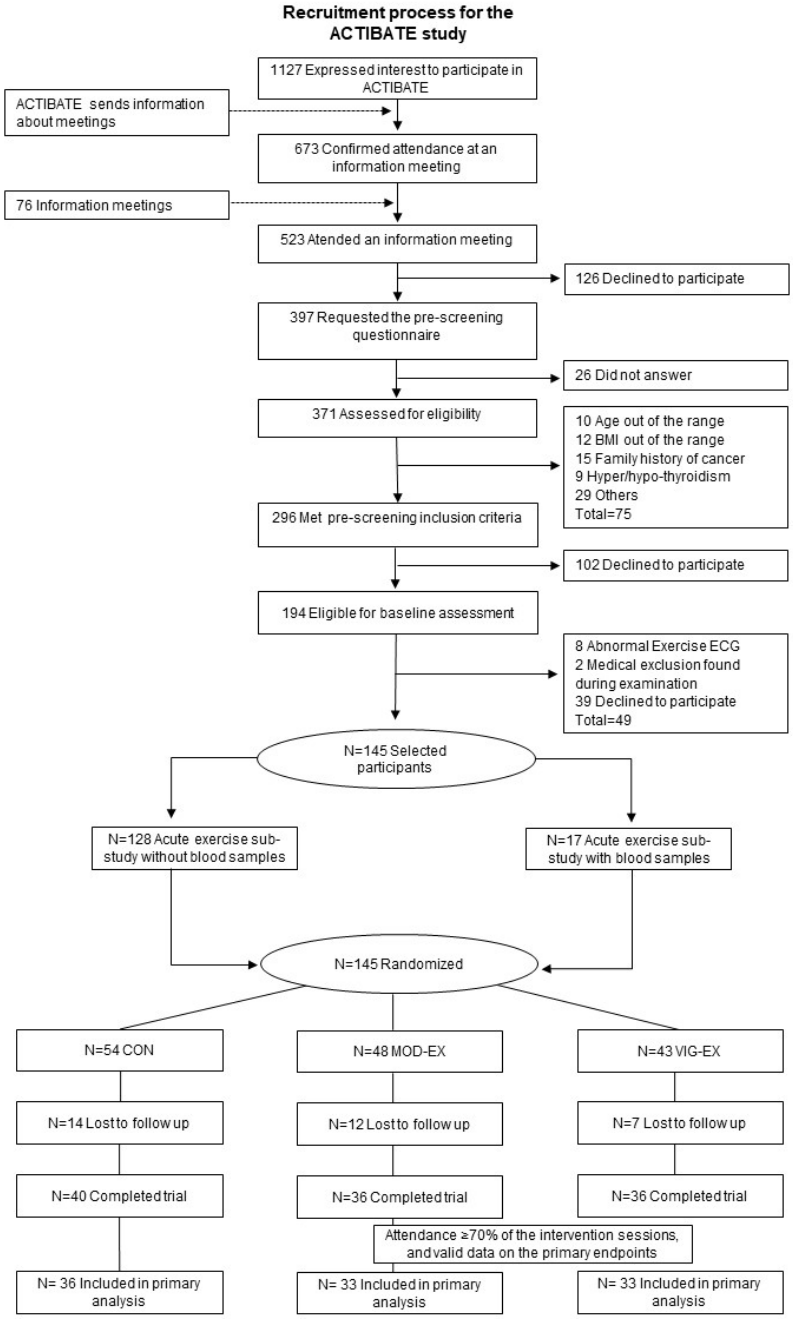

Figure S1. Study participants enrolment from the ACTIBATE study. *Abbreviations***:** BMI, body mass index; CON, control group; MOD-EX, moderate-intensity exercise group; VIG-EX: vigorous-intensity exercise group; ECG, electrocardiogram.

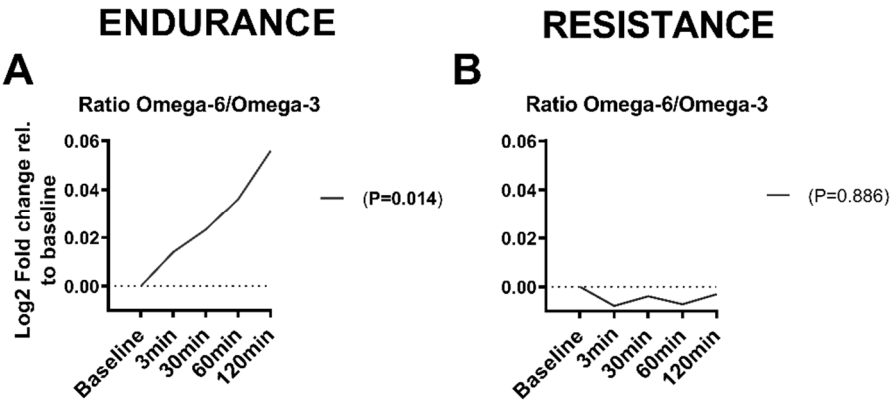

Figure S2. Acute endurance, but not resistance, exercise increases the ratio of omega-6/omega-3 oxylipins. Changes in the ration of omega-6/omega-3 oxylipins after acute endurance (A) and resistance (B) exercises. Each line represents the trajectory of the mean log2 fold change relative to baseline of the ratio omega-6/omega-3. P value obtained from repeated measures analyses of variance (ANOVA).