Prostaglandins, Leukotrienes and Essential Fatty Acids 202 (2024) 102642

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Original research article

Regular moderate physical activity potentially accelerates and strengthens both the pro-inflammatory and pro-resolving lipid mediator response after acute exercise stress

Linda Malan [a,*], Lizelle Zandberg [a], Cindy Pienaar [b,c], Arista Nienaber [a], Lize Havemann-Nel [a]

[a] Center of Excellence for Nutrition, North-West University, Potchefstroom, South Africa
[b] Physical Activity, Sport and Recreation Research Focus Area (PhASRec), Faculty of Health Sciences, North-West University, Potchefstroom, South Africa
[c] Department of Sport Studies, Faculty of Applied Sciences, Durban University of Technology, South Africa

ARTICLE INFO

Keywords:
Gene expression
Inflammation
Lipid mediator response
Post-exercise
Recreational runners
Resistance exercise

ABSTRACT

The PUFA-derived lipid mediator response shifts from pro-inflammatory to inflammation resolution over time and may be modified by regular moderate exercise. This pre-post-test study aimed to compare the expression of PTGES2 (COX2) and ALOX15 in leucocytes and the plasma 5- and 15-HETE, 18-HEPE and 17-HDHA responses after unaccustomed resistance exercise between 18–35-year-old male recreational runners (n = 18) and less-active controls (n = 15). One repetition maximum (1RM) was determined for squats, 45° leg presses and leg extensions. Subsequently three sets of 8–10 repetitions were performed at 80 % 1RM and blood collected over 72 hours. *PTGES2* and *ALOX15* expression changed over time in runners ($P = 0.016$, $P = 0.007$) but not controls ($P = 0.631$, $P = 0.539$). 5- and 15-HETE changed over time in runners ($P < 0.001$, $P = 0.022$), but not controls ($P = 0.457$, $P = 0.985$). 18-HEPE changed in runners and controls ($P < 0.001$, $P = 0.024$), 17-HDHA changed borderline in runners ($P = 0.076$). In conclusion, pro-inflammatory and inflammation-resolving lipid mediators may respond sooner and more robust in recreational runners than less-active controls after strenuous resistance exercise.

1. Introduction

Inflammation is a vital and normal part of the response to infection and injury [1]. Although the inflammatory response aims to protect against pathogens and restore tissue functioning it can also cause tissue damage and non-resolving chronic inflammation [1]. As such, many non-communicable diseases (NCDs), such as insulin resistance, atherosclerosis, cardiovascular diseases, neurodegeneration, and cancerous tumour growth are associated with oxidative stress and non-resolving inflammation [1–4]. Physical activity (PA) also activates the inflammatory response, due to the generation of reactive oxygen species (ROS) and may cause tissue damage, similar to physical injury [5,6]. Excessive non-resolving inflammation due to either continuous or once-off unaccustomed/strenuous high-intensity physical activity can negatively impact recovery through muscle soreness, reduced muscle strength, swelling, and injuries [6]. Inflammation resolution is, therefore, important in the disease and physical activity context to aid in the protection against inflammation-induced tissue damage [1]. There is increasing evidence that the capacity to resolve inflammation improves immune competence as well as the response to infection and injury [7,8].

The inflammatory response cascade involves the release of various intermediate metabolites, including lipid mediators released from muscle and immune cells [1]. Lipid mediators are bioactive intermediate metabolites derived from long-chain unsaturated fatty acids (LCPUFA), mostly from arachidonic acid (AA; 20:4n6), eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3) that are embedded in the phospholipid membrane of all cells [1,8]. Lipid mediators regulate inflammation by eliciting either a pro-inflammatory response via enzymatic activity of 2-cyclooxygenase (COX-2) or an inflammation-resolving response via 5- and 15-lipoxygenase (5, 15-LOX). The function of lipid mediators derived from n-6 LCPUFA (mainly AA) are mostly pro-inflammatory whilst those derived from n-3 LCPUFA (EPA and DHA) are inflammation-resolving [1,8] (Fig. 1). Among the first signalling events after infection or tissue injury is the release of pro-inflammatory lipid mediators that stimulate a series of

* Corresponding author.
E-mail address: linda.malan@nwu.ac.za (L. Malan).

https://doi.org/10.1016/j.plefa.2024.102642
Received 11 January 2024; Received in revised form 19 July 2024; Accepted 22 August 2024
Available online 23 August 2024

signalling cascades with the main aim to destroy the invading pathogens and repair the damaged tissue [1,8,9]. Consequently, in a time-dependent manner, lipid mediators derived from n-3 LCPUFA that signal the termination or resolution of inflammation are released [8,9]. Therefore, the pro-inflammatory lipid mediators, such as prostaglandins and leukotrienes and their intermediates, such as 5- and 15- hydroxytetraenoic acid (HETE) produced from AA can be measured in the periphery to assess inflammatory status. Likewise, specialised pro-resolving lipid mediators (SPMs), including resolvins (Rv), protectins, maresins and/or their intermediates such as 17-hydroxydocosahexaenoic acid (17-HDHA) and 18-hydroxyeicosapentaenoic acid (18-HEPE) can be measured to establish one's inflammation resolving capacity [8,9] (Fig. 1). Furthermore, associated inflammatory signalling gene expression responses, (e.g. nuclear factor kappa β [Nfκβ] and tumour necrosis factor α [TNFα]), lipid-mediator signalling and biosynthesis (e.g. COX and LOX), as well as oxidative stress–related enzymes (e.g. superoxide dismutase and glutathione peroxidase), can be detected in blood peripheral mononuclear cells (PBMC) [10].

Various strategies have been employed to improve inflammation resolution [1,8,9,11–13]. Firstly, the fatty acid composition of the cell membrane phospholipid bilayer serves as a reservoir for lipid mediator precursors and directly affects cell signalling. In turn, the membrane LCPUFA composition of the cells is dependent on endogenous biosynthesis of LCPUFA as well as dietary fatty acid intake of both alpha linolenic acid (ALA; 18:3n3) and linoleic acid (LA; 18:2n6) along with functional LCPUFA such as AA, DHA, and EPA. Therefore, dietary fatty acid intake and supplementation have been investigated as strategies to manipulate the lipid mediator response in both disease and physical activity contexts [1,8,10,14–18].

In addition, the magnitude of the inflammatory response to exercise is dependent on the intensity and duration of the exercise stimulus as well as the physical training status of the individual [19–22]. Exercise activates the inflammatory response via muscle tissue damage and/or oxidative stress, as a result of the increased production of reactive oxygen species (ROS) [5,12,13,22]. Regular moderate exercise training has been shown to produce an optimal amount of ROS to stimulate an adaptive response and increasing endogenous anti-oxidative capacity by up-regulation of antioxidant enzymes and molecules in skeletal muscle [5,21,23]. Endogenous enzymatic antioxidants include catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX), whereas non-enzymatic antioxidants include glutathione (GHS) [5,24,25]. Therefore, trained individuals have a lower level of oxidative damage (lipid peroxidation) than their less-active counterparts when subjected to the same relative workload [5]. Furthermore, exogenous antioxidants may also play an important role in the inflammatory response and intermediate metabolic pathways. Exogenous antioxidants are primarily dietary micronutrients which include vitamin C, vitamin E, zinc, vitamin D, selenium and carotenoids [24,25] often acting as important co-factors in the inflammatory response cascade [26]. Therefore, these exogenous (dietary antioxidant intake) and endogenous antioxidants may influence an individual's inflammation-resolving capacity.

Some evidence suggests that the protective effect of exercise against NCDs, may to some extent be ascribed to improving the capacity to resolve chronic inflammation [19,27]. Training status may thus be an important determinant of one's inflammation-resolving capacity. Limited data are available on the lipid mediator profiles in response to unaccustomed resistance exercise. Furthermore, it is not clear if moderate aerobic exercise training induces adaptation in terms of inflammation-resolving capacity, and whether exogenous antioxidants and fatty acid status are important contributors in the lipid mediator response. This study aimed to compare the *PTGES2* (COX2) and *ALOX15* expression and plasma 5- and 15-HETE, 18-HEPE and 17-HDHA responses after an unaccustomed resistance exercise challenge, of recreational male runners with less-active counterparts (controls).

2. Participants and methods

2.1. Participant recruitment and ethics statement

The study was conducted between September 2016 and March 2017. Male recreational runners (n= 18) and less active controls (n = 15) between the ages of 18 and 35 years volunteered and provided written informed consent to participate in the study (Table 1). The protocol was approved by the Health Research Ethics Committee of the North-West University (NWU-00061-16-A1). The study was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. The privacy and rights of the participants were ensured during the study. Participants were characterized as recreational runners if they had habitually performed three to five sessions of structured running or aerobic exercise per week (~30–60 min/session) during the last six months. Runners were excluded if they competed nationally or higher or performed more than five hours of structured exercise per week. Participants were included as less-active controls if they were not engaging in any regular (< 3 × 30–60 min sessions per week), structured exercise activity and/or physically demanding jobs that included manual labour during the last 6 months. Potential participants (runners and less active controls) were excluded if they had performed regular lower-body resistance training during the last six months, had an operation or medical procedure during the previous six months, reported to smoke, abusing drugs or alcohol, or were severely anaemic (Hb < 8 g/dL); had back pain or knee pain; had self-reported or previously diagnosed chronic conditions requiring the use of nonsteroidal anti-inflammatory drugs (NSAIDs) or cortisone

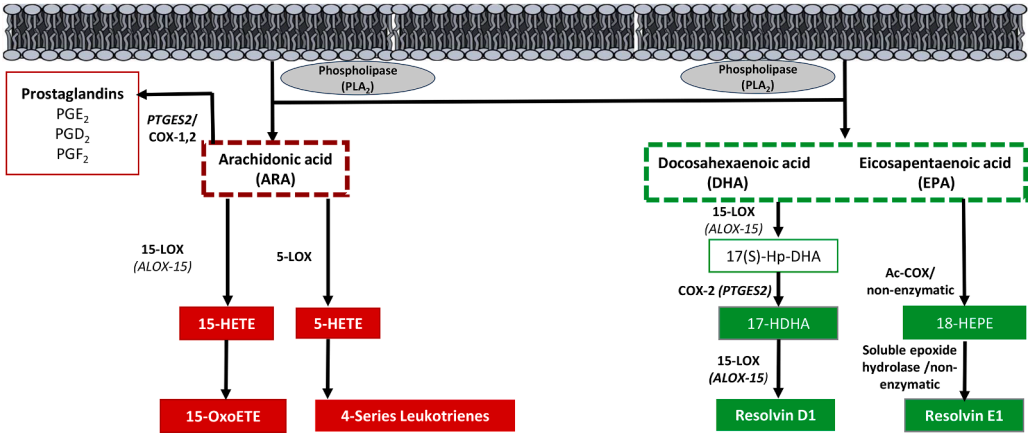

Fig. 1. Pro-inflammatory lipid mediator metabolism involving 5- and 15HETE intermediates and pro-resolving lipid mediator metabolism of resolvin D1 and E1.

Table 1
Participant characteristics of the recreational runners and less-active control men.

	Runners (n= 18)	Less-active controls (n= 15)	*P*-value[a]
Age (y)	24 (22, 29)[b]	21 (20, 26)	0.253
Activity			
Total activity score[c]	11 (9, 11)	7 (3, 8)	**<0.001**
Running	4 (3, 4)	1 (1, 1)	**<0.001**
Other structured exercise	4 (3, 4)	5 (1, 5)	0.740
Unstructured activities	3 (3, 3)	1 (1, 2)	**<0.001**
Anthropometry			
Height (m)	1.81 ± 0.06[d]	1.75 ± 0.07	**0.013**
Weight (kg)	80.3 ± 8.8	69.8 ± 11.6	**0.006**
BMI (kg/m^2)	24.6 ± 3.4	22.6 ± 3.0	0.131
Body fat percentage (%)	17.9 ± 9.3	14.7 ± 11.1	0.347
Biochemical and physiological parameters			
Haemoglobin (g/dL)	16.2 ± 1.4	15.9 ± 1.9	0.527
Glucose (mm/dL)	4.9 ± 0.4	4.8 ± 0.2	0.319
Systolic blood pressure (mmHg)	130 (120, 131)	130 (120, 135)	0.635
Diastolic blood pressure (mmHg)	80 (80, 80)	80 (70, 85)	0.532
Resting heart rate (bpm)	64 ± 9	65 ± 7	0.796
Oxidative stress			
Glutathione (μM)	1046 ± 117	1053 ± 117	0.869
Ferric reducing antioxidant power (μM)	473 ± 118	433 ± 86	0.286
Reactive oxygen species (arbitrary units)	60.2 ± 10.7	61.2 ± 11.5	0.811
One repetition maximum (1RM)			
Smith machine squat (kg)	113 ± 27	96 ± 14	**0.032**
45° Leg press (kg)	247 ± 64	218 ± 46	0.162
Knee extension (kg)	59 ± 8	50 ± 6	**0.002**

[a] Differences between runners and controls were examined with independent t-tests for normal data and Mann-Witney U tests for non-normally distributed data. $P < 0.05$ was considered significant.
[b] Non-normal data were reported as median (25th, 75th percentile).
[c] To calculate a total activity score from habitual aerobic physical activity, the sum of scores from running, other structured exercise (1. Never 2. Once a month 3. Once every one to two weeks 4. Two to three times a week 5. More than 4 times a week) and daily activity level [inactive (1), moderately active (2) or active (3)] was applied.
[d] Normal data were reported as mean ± SD. BMI, body mass index; bpm, beats per minute; mmHg, millimetre/mercury.

(systemic or inhalers); had an acute or chronic inflammatory disease, metabolic disease including type 1 and 2 Diabetes Mellitus, cardiovascular or clotting disease, previous hypertension or hypertension measured at the study medical screening.

2.2. Participant characteristics and strength testing

At screening/enrolment, candidates completed medical history forms, were assigned a participant number and underwent medical screening by assessing heart rate and blood pressure (Sphygomomameter (Tycos), finger prick fasting blood glucose (OneTouch Select, Johnson & Johnson) and haemoglobin (HemoCue® Hb 201+ System, Angelholm, Sweden); body temperature (ThermoScan IRT 3020/ IRT3520, Braun), height, weight (Seca 264, Hamburg, Germany) and body fat percentage (BF%) by air-displacement plethysmography with the BodPod® body compositions system (model 2000A, Life Measurement Instruments, Concord, CA, USA), shown in Fig. 2 and Table 1. Habitual physical activity during the past 6 months (aerobic and resistance exercise) was assessed with an administered questionnaire exploring how often they run, perform resistance exercise and/or other structured aerobic exercise sessions (e.g. walking, cycling, spinning and team sports) on a weekly and monthly basis. A score ranging from 1 to 5 was allocated based on a frequency from "never" to "more than 4 times per week". To calculate a total activity score from habitual aerobic physical activity, the sum of scores from running, other structured exercise and daily activity level [inactive (1), moderately active (2) or active (3)] was applied. Additionally, they completed a physical activity readiness questionnaire. Participants were familiarized with the exercises followed by multiple repetition maximum strength tests to determine the experimental exercise load [80 % of 1 repetition maximum (1 RM)] at least one week before the exercise study trial (exercise challenge) day. The maximal weight that subjects could lift for 3 to 6 repetitions with a Smith machine squat, 45° leg press and seated knee extension was determined. Subsequently, 1 RM was estimated using the Brzycki equation [1 RM= 100 X load rep/ (102.78 – 2.78 X_ reps completed)]. Participants were instructed to abstain from any unusual physical activity in the week before the experimental trial day.

2.3. Experimental exercise challenge protocol

Participants were asked to refrain from the consumption of any supplements, alcohol and NSAIDs from 1 week before the exercise challenge until the end of the study. They were also required to refrain from exercising for 2 days before the exercise challenge until the end of the study. Paracetamol was permissible. Participants received a standardized meal (57 % of the total energy from carbohydrates, 22 % from fat, and 21 % from protein) and a snack to consume the evening before the exercise challenge day. Participants again underwent medical tests and a physical activity readiness questionnaire before the exercise challenge started after an 8 to 10 h overnight fast. Still, in the fasted state, participants were then required to perform a 10 min warm-up, consisting of walking, and light cycling before the exercise challenge commenced. The exercise challenge consisted of three sets of 8 to 10 repetitions each on the Smith machine squat, 45° leg press and seated knee extension, at 80 % of the predetermined 1 RM. Exercises were performed sequentially in a circuit manner with participants resting for 3 min between each type of exercise and 1 minute between each set. Fig. 2 also illustrates the different time points of blood sampling before and following the exercise challenge. Immediately before and on completion of the exercise challenge, a blood sample was drawn (time point pre and 0 h in Fig. 2) and participants received a post-exercise snack and were instructed not to be active during a 2 h recovery period. Additional post-exercise blood samples were obtained 1 and 2 h after the cessation of exercise (time points 1 h and 2 h in Fig. 2). Participants consumed standardised meals and snacks for the rest of the day. Twenty-four hours after the exercise challenge another blood sample was drawn (time point 24 h in Fig. 2) after an overnight fast. No further dietary restrictions were required, but participants did not perform any exercise or consumed any supplements or NSAIDS until the last time point of blood sampling, collected 72 h after the exercise challenge in a non-fasted state (time point 72 h in Fig. 2).

2.4. Dietary intake control and analysis

A three-day dietary record, consisting of 2 weekdays and 1 weekend day, was completed by each participant prior to the exercise challenge to determine self-reported energy and nutrient intake. The 3-day dietary record data were analysed by the South African Medical Research Council (SAMRC). Portion sizes were converted to grams by registered dietitians/nutritionists using the SAMRC Food Quantities Manual [28]. All foods and drinks were coded according to the South African Food Composition Database [29]. Daily energy, macro-, and micronutrient intakes (antioxidant micronutrients and fatty acids) were calculated by the SAMRC by linking the coded and quantified dietary intake data to the most recent food composition database.

2.5. Sample collection

Venous blood samples were drawn into Heparin-coated, EDTA-coated, and trace element-free serum evacuated tubes (Becton

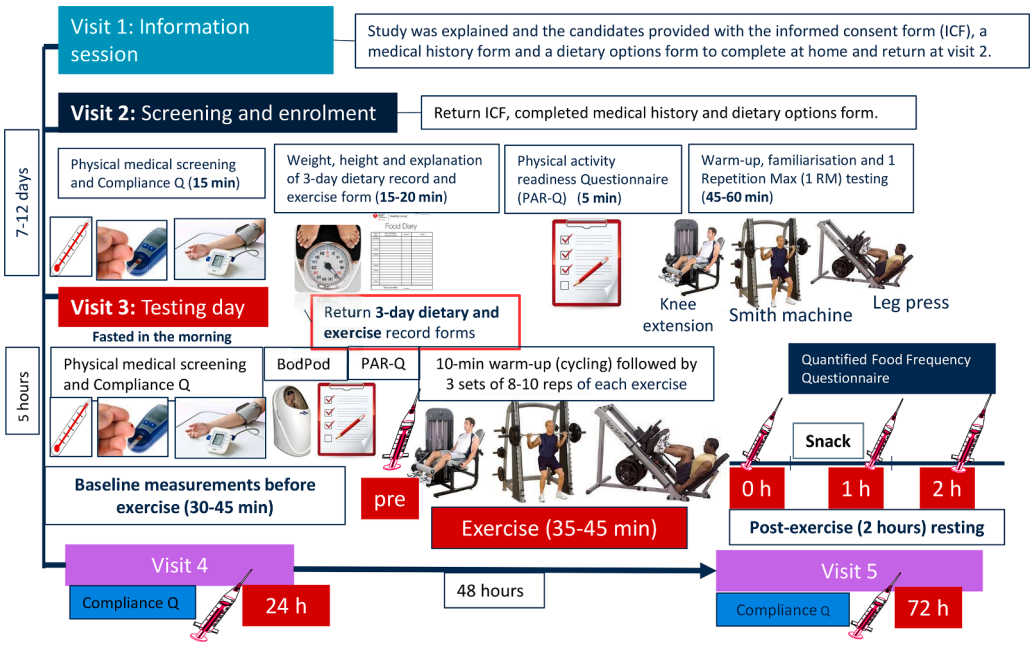

Fig. 2. Schedule of study activities.

Dickinson) via antecubital venipuncture. Blood was processed within 1 hour after blood sampling and plasma/serum was separated. Red blood cells were washed twice with normal saline and aliquots stored at -80 °C.

2.6. Lipid mediator analysis

Lipid mediators were extracted and analysed with liquid chromatography-tandem mass spectrometry (LCMSMS) [10]. The method was calibrated with external standards for 8-iso-prostaglandin (PG) F2-alpha, PGE2, PGB2, leukotriene B4, B5 and E4; 5-, 8-, 9-, 11-, 12- and 15-HETE; 5-, 8-, 9-, 11-, 12-, 15- and 18-HEPE, 17-HDHA, protectin D1, RvD1 and RvE1 (supplementary file). Lipid mediators were extracted from 1 ml plasma with solid-phase extraction (SPE) using Strata-X (Phenomenex, Torrance, United States of America) and normalised with 500 pg each of deuterated internal standard 12-HETE-d8. Extracted samples were analysed with an Agilent Technologies 6410 MSMS, coupled to an Infinity 1260 HPLC pump (Santa Clara, United States of America). The mass spectrometer has a mass range of 15–1650 m/z, minimum multiple reaction monitoring (MRM) dwell time of 5 ms with 10 MRMs and a maximum of 99 MRM transitions per time segment and was operated in negative electrospray ionisation mode. At least two transitions were monitored for each compound (one target and one qualifier), using a dynamic MRM method. The collision and fragment or voltages were optimised with flow injection analysis. The gas temperature and flow were set at 350 °C and 12 l/min respectively, and the capillary voltage at 4000 V. Chromatographic analysis was performed on a C18 column (Poroshell, 2.7 μ, 100 × 2.1 mm, Agilent Technologies) with a flow rate of 0.4 ml/min and a column temperature of 50 °C. Sample injections were performed with an Agilent G1367B autosampler. The sample chamber temperature was set at 5 °C and the injection volume at 15 μl, subsequent to mixing 5 μl of the sample (in acetonitrile) with 10 μl water in the autosampler, just before injection. The analysis was performed using an acetonitrile-based system with a flow program, by mixing two solvents (A and B) with programmed ratio changes for optimal separation of compounds. Solvent A was water / glacial acetic acid, 99:1(v/v) and solvent B was 100 % acetonitrile. Data were quantified with Masshunter B05 02, using external calibration for each compound and 12-HETE-d8 as internal standard to correct for losses and matrix effects during sample preparation and analysis. The qualifier limits were set between 80 % and 120 % of the original transition ratio. Data were provided as estimated means ± SE of internal standard-normalized arbitrary units.

2.7. Analysis of oxidative stress biomarkers

Oxidised glutathione (GSH) was measured with a spectrophotometric reduced/oxidized glutathione (GSH/GSSG) kit (GSH/GSSG-412™) from OXIS Research [30]. Reactive oxygen species (ROS) and ferric reducing antioxidant power (FRAP) were analysed with spectrophotometric methods [31,32].

2.8. Red blood cell total phospholipid fatty acids analysis

Total phospholipid fatty acids were analysed in red blood cells [33]. Phospholipids were extracted with chloroform: methanol (2:1 vol: vol, containing 0.01 % butylated hydroxytoluene). The phospholipids were separated from the neutral lipids by using thin-layer chromatography (silica gel 60 plates, 10 × 20 cm, Merck) and eluted with diethyl ether: petroleum, ether: acetic acid (30:90:1 vol: vol: vol) and trans-methylated with methanol: sulphuric acid (95:5 vol: vol) at 70 °C for 2 h to yield fatty acid methyl esters (FAMEs). The resulting FAMEs were analysed by using gas chromatography-tandem mass spectrometry on an Agilent Technologies 7890A Gas Chromatograph system, equipped with an Agilent Technologies 7000GC/MS triple quadrupole mass selective detector, with a HP88 capillary column (100 m x 0.25 mm x 0.20 μm, Agilent) by using helium as the carrier gas at a flow rate of 2.2 mL/min. The gas chromatography injector was held at a temperature of 270 °C, and the mass spectrometry source at 250 °C. The injection volume of the sample solution was 1 μL by using a split ratio of 1:20. The oven temperature was programmed from 50 °C to 170 °C at 30 °C/min, then from 170 °C to 215 °C at 2 °C/min, and it rose to 4 °C/min to 230 °C. After that, the temperature was held isothermally at 230 °C for 7 min. The total analysis time was 38.25 min. Mass spectrometry with 70 eV electron ionization was carried out in multiple reaction monitoring mode, with at least two transitions per compound. Quantification of FAME was done with Masshunter (B.06.00). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAMEs (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the

total concentration of all fatty acids identified in the sample [33], namely palmitic acid (16:0), palmitoleic acid (16:1n7), stearic acid (18:0), elaidic acid (18:1n9T), vaccenic acid (18:1n7T), oleic acid (18:1n9), vaccenic acid (18:1n7cis), linoleic acid (18:2n6), eicosanoic acid (20:0), gamma linolenic acid (18:3n6), alpha linolenic acid (18:3n3), eicosenoic acid (20:1n9), stearidonic acid (18:4n3), eicosadienoic acid (20:2n6), behenic acid (22:0), 20:3n3, dihomo-gamma-linolenic acid (20:3n6), mead acid (20:3n9), AA, erucic acid (22:1n9), EPA, lignoceric acid (24:0), docosatrienoic aicid (22:3n3), nervonic acid (24:1n9), adrenic acid (22:4n6), osbond acid (22:5n6), docosapentanoic acid (22:5n3), and DHA. Total saturated fatty acids was calculated as the sum of percentage of palmitic, stearic, eicosanoic, behenic and lignoceric acids; total monounsaturated fatty acids as the sum of palmitoleic, oleic, vaccenic, eicosenoic, erucic and nervonic acid; total n-3 PUFA as the sum of alpha linolenic, stearidonic, 20:3n3, EPA, docosatrienoic, docosapentanoic and DHA, total n-3 LCPUFA as the sum of stearidonic, 20:3n3, EPA, docosatrienoic, docosapentanoic and DHA; the total n-6 PUFA as the sum of linoleic, gamma linolenic acid, eicosadienoic, dihomo-gamma-linolenic, AA, adrenic and osbond acid; and total n-6 LCPUFA as the sum of gamma linolenic acid, eicosadienoic, dihomo-gamma-linolenic, AA, adrenic and osbond acid.

2.9. Gene expression

Total ribonucleic acid (RNA) was isolated from buffy coat stored in RNA*later*® (Ambion) with NucleoSpin® RNA Blood kit (Machery-Nagel). The yield, quality and integrity of the extracted total RNA samples were assessed using NanoDrop 1000 Spectrophotometer. Good quality total RNA with 260:280 ratio between 1.8-2 were used for first strand synthesis. Complementary DNA (cDNA) was prepared using LunaScript™ RT SuperMix Kit (New England Biolabs). Independent quantitative real-time PCR (qPCR) analysis was performed using TaqMan® hydrolysis probe chemistry-based gene expression assays for target genes *Arachidonate 15-Lipoxygenase* (*ALOX15, Hs00609608_m1) and Prostaglandin E Synthase 2 (PTGES2, Hs00228159_m1)*. Endogenous control genes *18S rRNA (Hs99999901_s1), and Beta Actin (ACTB; Hs99999903_m1),* were used with the Biorad CFX 96 instrument. Relative gene expression was assessed by means of $2^{-\Delta\Delta Ct}$ method described by Livak [34]. Target gene expression were normalized with the average of the two endogenous controls 18S rRNA and actin beta (ACTB). Relative mRNA expression is given as ΔCT and used for further statistical analysis.

2.10. Statistical analysis

Based on repeated-measures ANOVA with 6 time points, a sample size of 32 participants would provide 80 % power to detect an effect size of 0.40 with α= 0.05 and a correlation of 0.5 among repeated measures. Statistical analyses were performed with SPSS, version 27 (SPSS, Chicago, IL, USA). Data were assessed for normality with Q-Q plots and visual inspection of histograms. Normally distributed data are described as means and standard deviations and non-normally distributed data as medians and 25th, 75th percentiles. Non-normal data were transformed to attempt normality before analysis. Differences in participant characteristics, fatty acid composition and dietary intake between the runners and controls were examined with independent *t*-tests. Plasma lipid mediator responses and gene expression pre-exercise, directly post-exercise, and 1 h, 2 h and 24 h post-exercise, as well as at 72 h post-exercise for lipid mediator responses, were assessed with linear mixed models and Sidak corrections. Linear mixed models were used as all data available at each time point are utilised and thus missing data imputation is not necessary. Participants were treated as random effects and group and time were assessed separately as fixed effects. There was no effect for groups or group x time interaction. Significant covariate variables (any of weight, n-6 and/or n-3 LCPUFA status; dietary intake of n-3 PUFA, vitamin C, D, total carotenoid, selenium and/or zinc, as well as ROS and/or FRAP) were also treated as fixed effects. Participant length, dietary total n-6 PUFA and vitamin A intake and GSH tested not to be significant factors and were not included in the models. The effect size is the standardised difference between the means of two populations, where the square root of the sum of all covariance estimates was used as a measure of the standard deviation. Differences over time within the runner and control groups were assessed with paired t-tests. Type I error rate was set at 5 %.

3. Results

3.1. Participant characteristics

A total of 33 participants, with 18 in the runner group and 15 in the less-active control group were included in the study (Table 1). The runners had a higher total activity score, and more running sessions and other unstructured exercise sessions per week than the less-active controls (all $P < 0.001$). Runners were taller and heavier than the controls ($P= 0.013$ and $P= 0.006$, respectively). Runners had a higher 1 RM for squats on the Smith Machine ($P= 0.032$) and knee extension exercises ($P= 0.002$).

3.2. Red blood cell total phospholipid fatty acid composition

Table 2 shows that the red blood cell phospholipid EPA composition was higher in the runners than in the less-active controls ($P = 0.019$).

3.3. Daily dietary intake over three days during the study

The total n-3 LCPUFA, n-6 PUFA, n-6 LCPUFA, vitamin C, vitamin D,

Table 2
Red blood cell total phospholipid fatty acid composition.

Fatty acids (% of total fatty acids)	Runners (n= 18)	Less-active controls (n= 15)	*P*-value[a]
Docosahexaenoic acid (DHA)	4.0 ± 0.7[b]	3.7 ± 0.6	0.308
Eicosapentaenoic acid (EPA)	0.36 (0.32, 0.47)[c]	0.27 (0.21, 0.35)	**0.019**
Arachidonic acid (AA)	13.6 ± 1.7	14.0 ± 1.0	0.427
Saturated fatty acids (SFA)	43.5 (43.1, 44.0)	43.7 (43.5, 44.6)	0.364
Monounsaturated fatty acids (MUFA)	16.7 ± 1.3	15.9 ± 1.2	0.107
Total n-3 PUFA	6.7 ± 1.1	6.0 ± 0.9	*0.073*
Total n-3 LCPUFA	6.6 ± 1.1	6.0 ± 0.9	*0.076*
Total n-6 PUFA	32.1 ± 2.3	33.0 ± 2.0	0.239
Total n-6 LCPUFA	20.1 ± 2.1	21.6 ± 1.5	0.444
Total n-6: n-3 PUFA ratio	5.0 ± 1.0	5.6 ± 0.8	*0.063*
Total n-6: n-3 LCPUFA ratio	3.2 ± 0.7	3.5 ± 0.5	*0.076*

[a] Differences between runners and controls were examined with independent t-tests. P < 0.05 was considered significant. Non-normal data were log transformed before analysis.
[b] Normal data were reported as mean ± SD.
[c] Non-normal data were reported as median (25th, 75th percentile). LCPUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 3
Average daily dietary intake over three days during the study.

	Runners (n= 18)	Less-active controls (n= 15)	*P*-value[a]
Total energy (kJ)	10 567 ± 3073[b]	9 288 ± 3194	0.267
Total n-3 PUFA (g)	0.84 ± 0.31	0.58 ± 041	0.060
Total n-3 LCPUFA (g)	0.12 (0.07, 0.17)[c]	0.06 (0.02, 0.11)	**0.035**
Total n-6 PUFA (g)	19.2 (15.9, 36.5)	15.4 (5.5, 25.1)	**0.026**
Total n-6 LCPUFA (g)	0.25 (0.12, 0.35)	0.15 (0.08, 0.18)	**0.048**
Vitamin C (mcg)	46.0 (32.4, 68.7)	25.1 (14.2, 49.2)	**0.028**
Vitamin D (mg)	6.1 ± 3.0	3.5 ± 2.8	**0.023**
Vitamin E (mcg)	13.7 (11.0, 22.5)	10.1 (6.0, 12.9)	**0.009**
Carotenoids (mcg)	1487 (354, 2681)	576 (338, 1514)	0.519
Iron (mg)	15.0 ± 4.4	17.1 ± 8.4	0.391
Selenium (mcg)	78.3 ± 34.4	45.9 ± 29.1	**0.009**
Zinc (mg)	13.6 ± 4.9	14.4 ± 5.9	0.680

[a] Differences between runners and controls were examined with independent t-tests. P < 0.05 was considered significant. Non-normal data were log transformed before analysis.
[b] Normal data were reported as mean ± SD.
[c] Non-normal data were reported as median (25th, 75th percentile).

vitamin E and selenium dietary intake were higher in the runners compared to controls (*P*= 0.035, *P*= 0.026, *P*= 0.048, *P*= 0.028, *P*= 0.023, *P=0.009* and *P*= 0.009, respectively) as presented in Table 3.

3.4. Expression of COX2 and ALOX15 over 24 h after exercise challenge

The expression responses of both *PTGES2* gene encoding for COX2 and *ALOX15* encoding for 15-LOX were assessed over 24h after the exercise challenge. Fig. 3A and B show that *PTGES2* and *ALOX15* expression changed over time in runners (*P*= 0.016 and P= 0.007, respectively) but remained unchanged in the controls (*P* = 0.631 and *P*= 0.539, respectively). *PTGES2 (COX2)* expression in runners increased from pre-exercise to directly post-exercise (d= 0.5, *P*= 0.998) and then continue decreasing until 24 h post-exercise (d= 2.5, *P* = 0.023). In the controls, *PTGES2* decreased from pre- to post-exercise (d= 0.4, *P* = 0.999), then increased until 2 h post- (d= 0.4, *P* = 1.000), and decreased until 24 h post-exercise (d= 0.9, *P*= 0.983). *ALOX15* expression (Fig. 3B.) increased in runners from pre-exercise to post-exercise (d= 0.7, *P* = 0.839) and then decreased again until 24 h post-exercise (d= 1.6, *P*= 0.430). In the controls, *ALOX15* expression decreased from pre- to post-exercise (d= 0.4, *P* = 1.000) and then increased until 1 h post-exercise (d= 0.3, *P*= 1.000), and continued decreasing until 24 h (d= 1.0, *P*= 0.897).

3.5. Response of pro-inflammatory 5- and 15-HETE over 72 h after exercise challenge

Lipid mediators PGE2, PGE2, 5-, 8-, 9-, 11-, 12- and 15-HETE; 5-, 8-, 9-, 11-, 12-, 15- and 18-HEPE and 17-HDHA, were detected. Here we report on the COX and LOX metabolism, including 5- and 15-HETE and 18-HEPE, 17-HDHA (Fig. 1). Fig. 4 illustrates that the 5- and 15-HETE

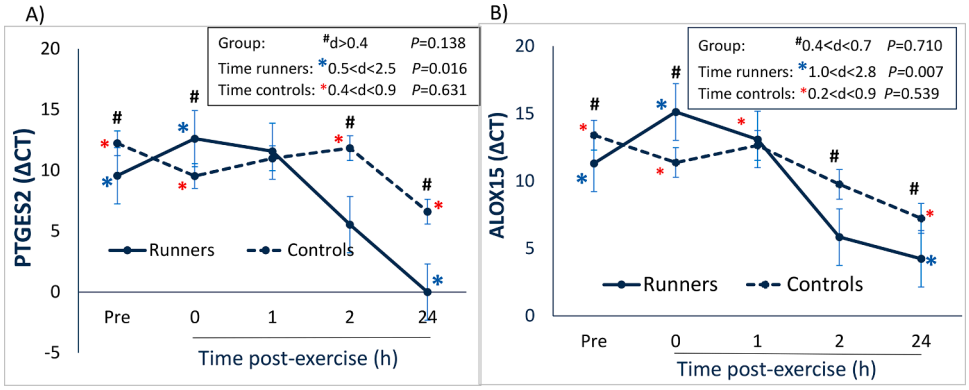

Fig. 3. Gene expression response to resistance exercise: A) PTGES2 (COX2), B) ALOX15 (LOX15). Runners n = 10, controls n = 8. Values are ΔCT normalized units. Difference between runners and controls and change over time in each group analysed with linear mixed models. Participants were treated as random effects and group and time as fixed effects. A) and B) adjusted for the daily average vitamin C intake of three days during the trial.

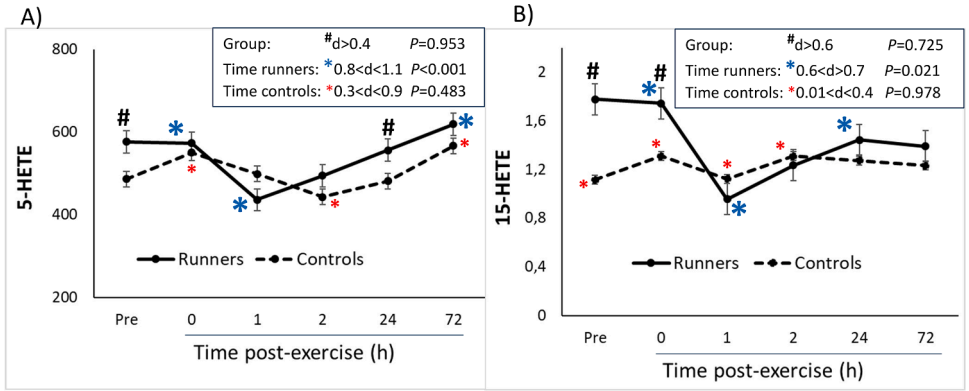

Fig. 4. Response of pro-inflammatory lipoxygenase intermediates to resistance exercise: A) 5-HETE, B) 15-HETE. Runners n = 15–17, controls n = 12–15). Values are estimated means ± SE of internal standard-normalized arbitrary units. Difference between runners and controls and change over time analysed with linear mixed models. Participants were treated as random effects and group and time as fixed effects. 5-HETE response (A) was adjusted for dietary total n-3 LCPUFA intake and 15-HETE response (B) for dietary total n-3 LCPUFA, total carotenoid and zinc intake of three days during the trial.

changed over time in runners ($P < 0.001$ and $P= 0.022$, respectively), but not in the controls ($P= 0.457$ and $P= 0.985$, respectively). In the runners, 5-HETE decreased from immediately (time 0 h) until 1 h post-exercise (d= 0.8, $P= 0.019$) and then increased again until 72 h post-exercise (d= 1.0, $P < 0.001$). Whereas, in the controls, 5-HETE increased from the start of exercise (d= 0.4, $P= 0.998$), then decreased from immediately (time 0 h) until 2 h post-exercise (d= 0.6, $P= 0.830$) followed with an increase again until 72 h post-exercise (d= 0.7, $P= 0.688$). In runners, 15-HETE decreased from directly post to 1 h post-exercise (d= 0.2, $P= 0.050$) and then increased until 24 h post-exercise (d= 0.2, $P= 0.442$). In controls 15-HETE decreased from directly post to 1 h post-exercise (d= 0.1, $P= 1.000$) and then increased until 2 h post-exercise (d= 0.1, $P= 1.000$).

3.6. Response of pro-resolving 18-HEPE and 17-HDHA over 72 h after exercise challenge

In Fig. 5, it is shown that 18-HEPE changed over time in both runners and controls ($P < 0.001$ and $P= 0.024$). 17-HDHA tended to change significantly over time in runners, but not in controls ($P= 0.076$ and $P= 0.703$). In runners, both 18-HEPE and 17-HDHA decreased from the start of exercise (Pre) until 1 h post-exercise (d= 7.4, $P = < 0.001$ and d= 0.7, $P= 0.419$, respectively), and increased again until 24 h post-exercise (d= 5.7, $P= 0.004$ and d= 0.6, $P= 0.789$). In controls, 18-HEPE decreased from the start of exercise (Pre) until 1 h after (d= 2.6, $P= 0.003$), and increased again until 72 h post-exercise (d= 3.2, $P= 0.037$), whereas 17-HDHA increased after 24 h until 72 h post-exercise (d= 0.3, $P= 0.194$).

4. Discussion and conclusion

This study showed that young men engaging in regular moderate aerobic exercise, in particular recreational running, potentially have a faster and more robust lipid mediator response, including inflammation resolution capacity, compared to their less-active controls. The faster response in runners, when adjusted for covariates, was evident from the early onset of *PTGES2* (COX2) and *ALOX15* gene expression from the start of exercise compared with the delayed onset of response only starting after termination of exercise in the less active control group. In addition, the more robust response in runners was also apparent from both the faster increase of pro-inflammatory intermediates (5-and 15-HETE) and decrease in pro-resolving intermediate (18-HEPE and 17-HDHA) concentrations, compared with the controls.

Cyclooxygenase-2 (COX-2) is an inducible enzyme, expressed at sites of inflammation, typical of a disease state. However, COX-2 is also expressed constitutively in areas not only associated with inflammation, but increasingly recognized for its homeostatic function in health [35]. Prostanoids (thromboxane A2 and prostaglandins) are produced via the enzymatic activity of COX-2. Several studies have found higher peripheral prostanoid concentrations for one to three days following resistance exercise [15,36–38]. These lipid mediators act together to collectively produce the cardinal signs of inflammation such as heat, swelling and pain [39]. Due to their inflammatory and immunoregulatory properties, they play a role in protein synthesis post-exercise, therefore, aiding recovery [40]. Evidence suggests that early prostaglandin synthesis post-exercise is essential for anabolic muscle recovery by commanding satellite cell proliferation and myogenesis and coordinating scavenger activity together with phagocytosis to remove debris and enhance tissue regeneration [41]. Corresponding with this, providing non-steroidal anti-inflammatory drugs during physical activity effectively inhibits COX-2 and blocks prostaglandin synthesis. Which in turn have been found to reduce muscle protein synthesis [41–44]. In this study, we found a faster peak in *COX-2* expression in the individuals engaging in regular moderate aerobic exercise compared to controls. Comparable to our results, other studies have also reported *COX-2* expression in peripheral blood mononuclear cells and muscle biopsies during recovery from exercise [39,45,46].

Previous research has shown higher lipid mediator concentrations following resistance exercise peaking from 1 -24 hours post-exercise and remaining high in the 2 to 3 days post-exercise depending on the specific lipid mediator [12,15,37,47]. Similarly, our findings supported these findings on pro-inflammatory lipid mediator intermediates. In this current study, the pro-inflammatory lipid mediator intermediates, 5- and 15-HETE, which are precursors for lipoxins and leukotrienes also peaked earlier in the runners compared with the non-runners [8]. Our results, therefore, suggest that regular exercise (such as running) induces adaptations including gene expression to promote an earlier *COX-2* expression and pro-inflammatory lipid mediator response in order to provoke inflammation resolution more quickly and effectively. Literature suggests a correlation between peak muscle soreness and peak pro-inflammatory lipid mediator concentrations post-exercise [37]. Therefore, a faster peak gene expression and lipid mediator response in individuals engaging in regular moderate aerobic exercise as found here, may lead to faster symptoms of inflammation, such as muscle soreness post-exercise, and subsequent faster recovery. Contrasting to the delayed onset and slower response in individuals not engaging in regular exercise [37]. Furthermore, as the pro-inflammatory lipid mediators are important in protein synthesis, it can also be hypothesised that muscle protein synthesis and recovery are initiated faster in the adapted muscle.

Inflammation resolution is initiated by the stimulus of the pro-inflammatory signal, an occurrence known as lipid mediator class

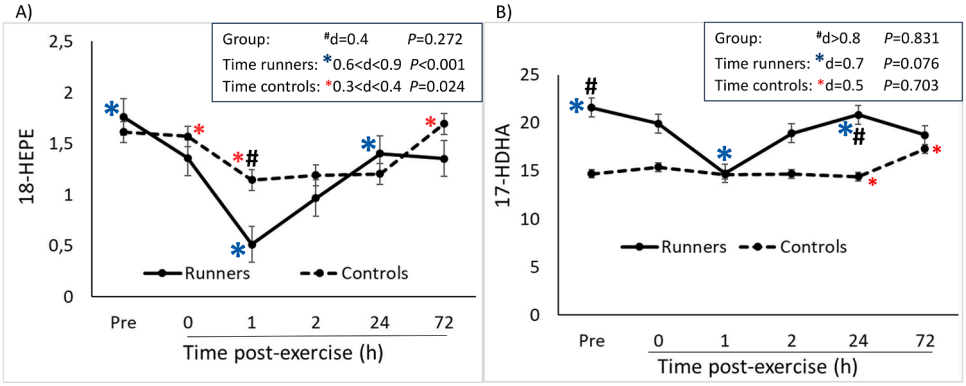

Fig. 5. Pro-resolving lipid intermediate 18-hydroxyeicosapentaenoic acid (18-HEPE) and 17-hydroxy-docosahexaenoic acid (17-HDHA) response before and after exercise challenge over time, runners n = 14–17 and controls n = 13–15. Values are means ± SE of internal standard-normalized arbitrary units. Difference between runners and controls and change over time analysed with linear mixed models. Participants were treated as random effects and group and time as fixed effects. There was no group x time interaction. 18-HEPE response (A) was adjusted for dietary vitamin D intake and 17-HDHA response (B) for weight, RBC total n-3 LCPUFA status and dietary total n-3 LCPUFA, total carotenoid and zinc intake over three days during the trial.

switching. This may explain the more robust response of pro-resolving intermediates in runners, who presents with a faster pro-inflammatory lipid mediator intermediate response [8]. As a result, in the runners, the pro-resolving lipid mediator intermediates, 18-HEPE and 17-HDHA started to decrease immediately post-exercise, but not in the less-active controls. In addition, the pro-resolving lipid intermediate 17-HDHA only started to increase in controls from 24 h, also indicating a later response in the less-active controls. Higher EPA and DHA intake promote n-3 PUFA derived lipid mediator production which in turn contribute to the regulation of inflammation, enhancement of muscle protein synthesis and regeneration, and lowering of signs of muscle damage induced by eccentric exercise e.g., muscle soreness [48–52]. This implies that the bioactive lipid signalling and lipid mediator response in the runners may contribute to faster recovery. Even though no data was collected in the current study reporting on the clinical signs of muscle damage this observation agrees with the notion that less-active or sedentary individuals usually take longer to recover from muscle soreness after exercise than individuals engaging in regular exercise [6,40].

When considering the oxidative stress response, the results of the current study may seem counterintuitive. Since the inflammatory response is partially dependent on oxidative stress, which is higher in sedentary individuals after an acute unaccustomed exercise bout, one may also expect a more pronounced inflammatory response in less-active controls [23]. However, there was no difference in oxidative stress markers between the runners and the less-active controls in the present study. Therefore, the difference in lipid mediator response may be independent of oxidative stress in this population of young men. In addition, although not the focus of this study, it is noteworthy to acknowledge that, as expected, total red blood cell n-3 LCPUFA and total n-3 LCPUFA dietary intake in the 3 days before the exercise stimulus, were significant predictors of the lipid mediator response post exercise. Additionally, vitamin C and D, total carotenoid, zinc and selenium intake over the three days prior to the exercise were also significant predictors, agreeing with other studies investigating the role of these nutrients and an antioxidant rich diet on the inflammatory response post-exercise [53–55].

It should be acknowledged that the small samples size is not ideal and that these results need to be confirmed by studies with a larger sample size to be generalisable to the population of young men. Also, data on the clinical symptoms of muscle damage could have been of value in unravelling some of the speculated mechanisms of action.

In conclusion, this study added to the current body of evidence that that regular moderate exercise like recreational running may lead to adaptations that promote a faster and more robust pro-inflammatory and pro-resolving lipid mediator response to unaccustomed strenuous exercise comparing to the delayed response of less-active controls. This has a practical implication for faster recovery following intense training.

Funding

This work was supported by personal incentive funding from the National Research Foundation of South Africa (Lize Havemann-Nel). The funder had no involvement in the study design, data collection, analysis and interpretation, writing of the report or the decision to submit the article for publication.

CRediT authorship contribution statement

Linda Malan: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lizelle Zandberg:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Cindy Pienaar:** Writing – review & editing, Methodology, Investigation. **Arista Nienaber:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Lize Havemann-Nel:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of interest

The authors have no conflict of interest.

Acknowledgements

We would like to thank Sr Alida Anthony and Sr Chrissie Lessing of the Este Vorster Research Facility at the North-West University as well as Dr JE Kotze for their medical supervision during the study. Thank you to the master's students Ms Johnine Viljoen and Erika Bezuidenhout for your participation in the data collection. We thank Mrs Cecile Cooke and Ms Katlego Kekana for their assistance with the laboratory analyses and Dr Chrisna Botha-Ravyse and Mr Johann Sauer for their role in overseeing the exercise protocol, and Prof Suria Ellis for statistics consultation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2024.102642.

References

[1] P.C. Calder, n-3 PUFA and inflammation: from membrane to nucleus and from bench to bedside, Proc. Nutr. Soc. 79 (2020) 404–416.
[2] G.K. Hansson, Inflammation, atherosclerosis, and coronary artery disease, N. Engl. J. Med. 352 (2005) 1685–1695.
[3] F. Novellino, V. Saccà, A. Donato, P. Zaffino, M.F. Spadea, M. Vismara, B. Arcidiacono, N. Malara, I. Presta, G. Donato, Innate immunity: a common denominator between neurodegenerative and neuropsychiatric diseases, Int. J. Mol. Sci. 21 (2020) 1115.
[4] F. Colotta, P. Allavena, A. Sica, C. Garlanda, A. Mantovani, Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability, Carcinogenesis 30 (2009) 1073–1081.
[5] S.K. Powers, W.B. Nelson, M.B. Hudson, Exercise-induced oxidative stress in humans: cause and consequences, Free Rad. Biol. Med. 51 (2011) 942–950.
[6] J.M. Peake, O. Neubauer, P.A. Della Gatta, K. Nosaka, Muscle damage and inflammation during recovery from exercise, J. Appl. Physiol. 122 (2017) 559–570.
[7] J.N. Fullerton, D.W. Gilroy, Resolution of inflammation: a new therapeutic frontier, Nat. Rev. Drug Discov. 15 (2016) 551–567.
[8] C.N. Serhan, Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms, FASEB J. 31 (2017) 1273–1288.
[9] C.N. Serhan, N.A. Petasis, Resolvins and protectins in inflammation resolution, Chem. Rev. 111 (2011) 5922–5943.
[10] L. Malan, J. Baumgartner, L. Zandberg, P. Calder, C. Smuts, Iron and a mixture of DHA and EPA supplementation, alone and in combination, affect bioactive lipid signalling and morbidity of iron deficient South African school children in a two-by-two randomised controlled trial, Prostaglandins Leukotrienes Essential Fatty Acids 105 (2016) 15–25.
[11] M. Tomczyk, Z. Jost, M. Chroboczek, R. Urbański, P.C. Calder, H.L. Fisk, M. Sprengel, J. Antosiewicz, Effects of 12 weeks of omega-3 fatty acid supplementation in long-distance runners, Med. Sci. Sports Exerc., (9900) 10.1249/MSS.0000000000003038.
[12] J.F. Markworth, L. Vella, B.S. Lingard, D.L. Tull, T.W. Rupasinghe, A.J. Sinclair, K. R. Maddipati, D. Cameron-Smith, Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment, Am. J. Physiol. Regul. Integr. Compar. Physiol. 305 (2013) R1281–R1296.
[13] J.F. Markworth, L.A. Brown, E. Lim, C. Floyd, J. Larouche, J.A. Castor-Macias, K. B. Sugg, D.C. Sarver, P.C. Macpherson, C. Davis, Resolvin D1 supports skeletal myofiber regeneration via actions on myeloid and muscle stem cells, JCI Insight 5 (2020).
[14] R.J. Bloomer, D.E. Larson, K.H. Fisher-Wellman, A.J. Galpin, B.K. Schilling, Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: a randomized, placebo controlled, cross-over study, Lipids Health Dis. 8 (2009) 1–12.
[15] B. Tartibian, B.H. Maleki, A. Abbasi, Omega-3 fatty acids supplementation attenuates inflammatory markers after eccentric exercise in untrained men, Clin. J. Sport Med. 21 (2011) 131–137.
[16] M. Martorell, X. Capó, A. Sureda, J.M. Batle, I. Llompart, E. Argelich, J.A. Tur, A. Pons, Effect of DHA on plasma fatty acid availability and oxidative stress during training season and football exercise, Food Funct. 5 (2014) 1920–1931.
[17] X. Capó, M. Martorell, A. Sureda, I. Llompart, J.A. Tur, A. Pons, Diet supplementation with DHA-enriched food in football players during training season enhances the mitochondrial antioxidant capabilities in blood mononuclear cells, Eur. J. Nutr. 54 (2015) 35–49.

[18] D. Buonocore, M. Verri, A. Giolitto, E. Doria, M. Ghitti, M. Dossena, Effect of 8-week n-3 fatty-acid supplementation on oxidative stress and inflammation in middle-and long-distance running athletes: a pilot study, J. Int. Soc. Sports Nutr. 17 (2020) 55.
[19] B.K. Pedersen, M.A. Febbraio, Muscle as an endocrine organ: focus on muscle-derived interleukin-6, Physiol. Rev. 88 (2008) 1379–1406.
[20] J.C. Rosa Neto, F.S.d. Lira, M. De Mello, R.V.T. Santos, Importance of exercise immunology in health promotion, Amino Acids. 41 (2011) 1165–1172.
[21] F. Seifi-Skishahr, A. Damirchi, M. Farjaminezhad, P. Babaei, Physical training status determines oxidative stress and redox changes in response to an acute aerobic exercise, Biochem. Res. Int. 2016 (2016).
[22] M.J. Jackson, A. Vasilaki, A. McArdle, Cellular mechanisms underlying oxidative stress in human exercise, Free Rad. Biol. Med. 98 (2016) 13–17.
[23] S. Falone, A. Mirabilio, A. Pennelli, M. Cacchio, A. Di Baldassarre, S. Gallina, A. Passerini, F. Amicarelli, Differential impact of acute bout of exercise on redox- and oxidative damage-related profiles between untrained subjects and amateur runners, Physiol. Res. 59 (2010) 953.
[24] M.L. Urso, P.M. Clarkson, Oxidative stress, exercise, and antioxidant supplementation, Toxicology 189 (2003) 41–54.
[25] P. Steinbacher, P. Eckl, Impact of oxidative stress on exercising skeletal muscle, Biomolecules 5 (2015) 356–377.
[26] A.B. Jena, R.R. Samal, N.K. Bhol, A.K. Duttaroy, Cellular Red-Ox system in health and disease: the latest update, Biomed. Pharmacother. 162 (2023) 114606.
[27] A.M. Minihane, S. Vinoy, W.R. Russell, A. Baka, H.M. Roche, K.M. Tuohy, J. L. Teeling, E.E. Blaak, M. Fenech, D. Vauzour, Low-grade inflammation, diet composition and health: current research evidence and its translation, Br. J. Nutr. 114 (2015) 999–1012.
[28] SAFOODS, SAMRC Food Quantities Manual for South Africa, 3rd ed., South African Medical Research Council, Cape Town, 2018.
[29] SAFOODS, SAMRC Food Composition Tables for South Africa, 5th ed., South African Medical Research Council, Cape Town, 2017.
[30] C. Janse van Rensburg, E. Erasmus, D.T. Loots, W. Oosthuizen, J.C. Jerling, H. S. Kruger, R. Louw, M. Brits, F.H. van der Westhuizen, Rosa roxburghii supplementation in a controlled feeding study increases plasma antioxidant capacity and glutathione redox state, Eur. J. Nutr. 44 (2005) 452–457.
[31] F. Benzie, I F, & Strain, JJ (1996), The ferric reducing ability of plasma (FRAP) as a measure.
[32] I. Hayashi, Y. Morishita, K. Imai, M. Nakamura, K. Nakachi, T. Hayashi, High-throughput spectrophotometric assay of reactive oxygen species in serum, Mut. Res./Genet. Toxicol. Environ. Mutagen. 631 (2007) 55–61.
[33] J. Baumgartner, C.M. Smuts, L. Malan, J. Kvalsvig, M.E. van Stuijvenberg, R. F. Hurrell, M.B. Zimmermann, Effects of iron and n-3 fatty acid supplementation, alone and in combination, on cognition in school children: a randomized, double-blind, placebo-controlled intervention in South Africa, Am. J. Clin. Nutr. 96 (2012) 1327–1338.
[34] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method, Methods 25 (2001) 402–408.
[35] N.S. Kirkby, M.V. Chan, A.K. Zaiss, E. Garcia-Vaz, J. Jiao, L.M. Berglund, E. F. Verdu, B. Ahmetaj-Shala, J.L. Wallace, H.R. Herschman, Systematic study of constitutive cyclooxygenase-2 expression: Role of NF-κB and NFAT transcriptional pathways, Proc. Natl Acad. Sci. 113 (2016) 434–439.
[36] E. Dousset, J. Avela, M. Ishikawa, J. Kallio, S. Kuitunen, H. KYRÚLàINEN, V. Linnamo, P.V. Komi, Bimodal recovery pattern in human skeletal muscle induced by exhaustive stretch-shortening cycle exercise, Med. Sci. Sports Exerc. 39 (2007) 453–460.
[37] M.C. Uchida, K. Nosaka, C. Ugrinowitsch, A. Yamashita, E. Martins Jr, A. S. Moriscot, M.S. Aoki, Effect of bench press exercise intensity on muscle soreness and inflammatory mediators, J. Sports Sci. 27 (2009) 499–507.
[38] D. Boatwright, R. Byrd, M. Mangum, Relationship between serum prostaglandin formation, creatine kinase activity, and ratings of perceived soreness, Res. Sports Med. Int. J. 2 (1991) 85–88.
[39] S.Y. Kim, T.W. Jun, Y.S. Lee, H.K. Na, Y.J. Surh, W. Song, Effects of exercise on cyclooxygenase-2 expression and nuclear factor-κB DNA binding in human peripheral blood mononuclear cells, Ann. N. Y. Acad. Sci. 1171 (2009) 464–471.
[40] J.F. Markworth, K.R. Maddipati, D. Cameron-Smith, Emerging roles of pro-resolving lipid mediators in immunological and adaptive responses to exercise-induced muscle injury, Exerc. Immunol. Rev. 22 (2016).
[41] U.R. Mikkelsen, I.C. Helmark, M. Kjær, H. Langberg, Prostaglandin synthesis can be inhibited locally by infusion of NSAIDS through microdialysis catheters in human skeletal muscle, J. Appl. Physiol. 104 (2008) 534–537.
[42] T.A. Trappe, F. White, C.P. Lambert, D. Cesar, M. Hellerstein, W.J. Evans, Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis, Am. J. Physiol. Endocrinol. Metab. 282 (2002) E551–E556.
[43] U.R. Mikkelsen, H. Langberg, I.C. Helmark, D. Skovgaard, L.L. Andersen, M. Kjaer, A.L. Mackey, Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise, J. Appl. Physiol. 107 (2009) 1600–1611.
[44] A.L. Mackey, M. Kjaer, S. Dandanell, K.H. Mikkelsen, L. Holm, S. Døssing, F. Kadi, S.O. Koskinen, C.H. Jensen, H.D. Schrøder, The influence of anti-inflammatory medication on exercise-induced myogenic precursor cell responses in humans, J. Appl. Physiol. 103 (2007) 425–431.
[45] C.C. Carroll, D.T. O'Connor, R. Steinmeyer, J.D. Del Mundo, D.R. McMullan, J. A. Whitt, J.E. Ramos, R.J. Gonzales, The influence of acute resistance exercise on cyclooxygenase-1 and-2 activity and protein levels in human skeletal muscle, Am. J. Physiol. Regul. Integr. Compar. Physiol. 305 (2013) R24–R30.
[46] E.M. Weinheimer, B. Jemiolo, C.C. Carroll, M.P. Harber, J.M. Haus, N.A. Burd, J. K. Lemoine, S.W. Trappe, T.A. Trappe, Resistance exercise and cyclooxygenase (COX) expression in human skeletal muscle: implications for COX-inhibiting drugs and protein synthesis, Am. J. Physiol. Regul. Integr. Compar. Physiol. 292 (2007) R2241–R2248.
[47] L. Hirose, K. Nosaka, M. Newton, A. Laveder, M. Kano, J. Peake, K. Suzuki, Changes in inflammatory mediators following eccentric exercise of the elbow flexors, Exerc. Immunol. Rev. 10 (2004) 20.
[48] C.J. Gerling, K. Mukai, A. Chabowski, G.J. Heigenhauser, G.P. Holloway, L. L. Spriet, S. Jannas-Vela, Incorporation of omega-3 fatty acids into human skeletal muscle sarcolemmal and mitochondrial membranes following 12 weeks of fish oil supplementation, Front. Physiol. 10 (2019) 348.
[49] P.C. Calder, Eicosapentaenoic and docosahexaenoic acid derived specialised pro-resolving mediators: Concentrations in humans and the effects of age, sex, disease and increased omega-3 fatty acid intake, Biochimie 178 (2020) 105–123.
[50] G.I. Smith, P. Atherton, D.N. Reeds, B.S. Mohammed, D. Rankin, M.J. Rennie, B. Mittendorfer, Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia–hyperaminoacidaemia in healthy young and middle-aged men and women, Clin. Sci. 121 (2011) 267–278.
[51] Y. Tsuchiya, K. Yanagimoto, K. Nakazato, K. Hayamizu, E. Ochi, Eicosapentaenoic and docosahexaenoic acids-rich fish oil supplementation attenuates strength loss and limited joint range of motion after eccentric contractions: a randomized, double-blind, placebo-controlled, parallel-group trial, Eur. J. Appl. Physiol. 116 (2016) 1179–1188.
[52] S. Jeromson, I. Mackenzie, M.K. Doherty, P.D. Whitfield, G. Bell, J. Dick, A. Shaw, F. Rao, S. Ashcroft, A. Philp, Lipid remodelling and an altered membrane proteome may drive the effects of EPA and DHA treatment on skeletal muscle glucose uptake and protein accretion, AJP-Endocrinol. Metab. (Endocrinol. Metab.) 314 (2018) e605–e619.
[53] S. Bohlooli, F. Rahmani-Nia, P. Babaei, B. Nakhostin-Roohi, Influence of vitamin C moderate dose supplementation on exercise-induced lipid peroxidation, muscle damage and inflammation, Medicina dello Sport 65 (2012) 187–197.
[54] A. Gmiat, J. Mieszkowski, K. Prusik, K. Prusik, J. Kortas, A. Kochanowicz, A. Radulska, M. Lipiński, M. Tomczyk, J. Jaworska, Changes in pro-inflammatory markers and leucine concentrations in response to Nordic Walking training combined with vitamin D supplementation in elderly women, Biogerontology. 18 (2017) 535–548.
[55] A.E. Koivisto, T. Olsen, I. Paur, G. Paulsen, N.E. Bastani, I. Garthe, T. Raastad, J. Matthews, R. Blomhoff, S.K. Bøhn, Effects of antioxidant-rich foods on altitude-induced oxidative stress and inflammation in elite endurance athletes: A randomized controlled trial, PLoS. One 14 (2019) e0217895.