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Arachidonic acid supplementation modulates blood and skeletal muscle lipid profile with no effect on basal inflammation in resistance exercise trained men

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ABSTRACT

Arachidonic acid (ARA), an omega-6 polyunsaturated fatty acid (PUFA), is the metabolic precursor to the eicosanoid family of lipid mediators. Eicosanoids have potent pro-inflammatory actions, but also act as important autocrine/paracrine signaling molecules in skeletal muscle growth and development. Whether dietary ARA is incorporated into skeletal muscle phospholipids and the resulting impact on intramuscular inflammatory and adaptive processes in-vivo is not known. In the current study, resistance trained men (≥1 year) received dietary supplementation with 1.5 g/day ARA (n = 9, 24 ± 1.5 years) or placebo (n = 10, 26 ± 1.3 years) for 4-weeks while continuing their normal training regimen. Plasma and *vastus lateralis* muscle biopsies were collected in an overnight fasted state at baseline and week 4. ARA supplementation increased plasma content of ARA and gamma-linolenic acid, while decreasing relative abundance of linoleic acid, eicosapentaenoic acid, and dihomo-gamma-linolenic acid. In skeletal muscle, ARA and dihomo-gamma-linolenic acid content increased, whereas alpha-linolenic-acid was reduced. Compared to placebo, ARA supplementation reduced circulating platelet and monocyte number, and decreased the mRNA expression of the immune cell surface markers; neutrophil elastase/CD66b and interleukin 1-beta, in peripheral blood mononuclear cells. In muscle, ARA supplementation increased mRNA expression of the myogenic regulatory factors; MyoD and myogenin, but had no effect on a range of immune cell markers or inflammatory cytokines. These data show that dietary ARA supplementation can rapidly and safely modulate plasma and muscle fatty acid profile and promote myogenic gene expression in resistance trained men, without a risk of increasing basal systemic or intramuscular inflammation.

1. Introduction

Arachidonic acid (ARA) is a long-chain omega-6 (n-6) polyunsaturated fatty acid (PUFA) which is an important metabolic precursor in the synthesis of bioactive eicosanoid lipid mediators [1]. ARA can be synthesized *de novo* from the dietary essential n-6 precursor linoleic acid via a series of elongation and desaturation reactions [2], or ingested preformed in dietary sources including meat, poultry, fish and eggs [3]. Linoleic acid is by far the most highly consumed PUFA in the western diet (~14.8 g/d or ~6.7% of energy [4]) and in the complete absence of ingestion of other n-6 PUFA is the sole source of cellular ARA [5,6]. Nevertheless, in adults consuming western-type diets, conversion of dietary linoleic acid to ARA in humans is extremely inefficient (e.g. ~0.2% at ~6% energy as n-6 PUFA) [7], and large changes in linoleic acid intake appear to have little impact on plasma/serum ARA content [8–10]. By comparison, ARA is typically consumed at ~0.15 g/d in the Western diet [11,12] and the ARA content of plasma/serum lipids is highly sensitive to changes in dietary ARA intake [8]. Therefore, ingestion of preformed sources of ARA seems to be the primary determinant of ARA availability in humans not deficient in linoleic acid [8,13–17].

Since ARA is the precursor to the prostaglandins and leukotrienes,

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which play an important role in the local inflammatory response, it has been hypothesized that excess dietary ARA intake may promote inflammation [18–21]. It has also recently been suggested that excess dietary n-6 PUFA might increase inflammation via the formation of specific oxylipin metabolites of linoleic acid itself [22–24]. Other studies have found that higher overall n-6 PUFA consumption is associated with unaltered or lowered inflammation [25–29], improved blood cholesterol profile [30–32] and a reduced risk of coronary heart disease [33–35]). Less is known regarding the role of ingestion of ARA itself, but a small number of short term human trials have reported that dietary intake of ARA up to ~1.5 g/day appears to have little or no effect on circulating inflammatory markers [36–44]. Further evidence that an elevated tissue ARA content might not necessarily promote inflammation comes from observational studies in which serum ARA content has actually been found to be inversely associated with blood inflammatory markers [45].

In addition to their role as inflammatory mediators, ARA and its metabolites play important physiological roles as local signaling molecules in skeletal muscle [1,46]. In myogenic cells cultured in-vitro, supplementation of the culture media with ARA can promote cell growth [47–50], improve insulin sensitivity [51–53], and protect against saturated fatty acid induced lipotoxicity [53] and inflammatory signaling [51,54]. In a recent human randomized controlled trial, dietary ARA supplementation (1.5 g/day for 8 weeks) was reported to augment gains in muscle mass and strength in men participating in a resistance exercise training program [55]. Whether dietary ARA is incorporated into blood and skeletal muscle phospholipids and the resulting impact on systemic and intramuscular inflammation in individuals participating in resistance exercise training is not known.

The present study aimed to determine the effect of 4-weeks of dietary supplementation with 1.5 g/day ARA on blood and skeletal muscle tissue fatty acid composition and whether changes in tissue ARA abundance would modulate basal inflammation. It was hypothesized that supplemental ARA would be incorporated into plasma and muscle lipids, but that this would not result in an increased state of systemic or intramuscular inflammation in healthy young men participating in regular resistance exercise training.

2. Materials and methods

2.1. Participants

Twenty healthy young active male participants (18–35 years of age) were recruited to participate in this study. Participants were randomized to receive dietary supplementation with 1.5 g/day ARA (n = 11) or placebo (n = 10) for a duration of 4-weeks (Fig. 1). Inclusion criteria required that participants were currently participating in a resistance exercise training program for ≥1 year which included at least one leg based training session per week. Any existing injuries, cardiovascular, musculoskeletal, or metabolic disease, and use of medication or performance enhancing drugs (e.g. anabolic steroids) excluded participants from participation. Regular use of common dietary supplements (e.g. vitamins/minerals (placebo group n = 1, ARA group n = 1), protein/amino acids (placebo group n = 6, ARA group n = 5) and creatine (placebo group n = 2 ARA group n = 3) was not grounds for exclusion. However, any participants who reported recent consumption of any fatty acid supplements (e.g. fish oil) were required to undergo a ≥1 month wash out period (based on [44]) prior to participation in the study (placebo group n = 2, ARA group n = 2). One participant that was randomized to the ARA group sustained a back injury prior to follow up testing and another withdrew from the study due to a tooth ache that required NSAID treatment. Thus, results are presented for n = 10 in the placebo group and n = 9 in the ARA group (Fig. 1). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants/patients were approved by the Northern Health and Disability Ethics Committee (New Zealand) (14/NTA/147). Before commencement of the study the experimental protocol was explained to the participants and informed written consent to participate was obtained from all participants. The clinical trial was registered with the Australian New Zealand Clinical Trial Registry (ANZCTR) (ACTRN12615000710527).

2.2. Experimental design

This was a double blind randomized placebo controlled parallel arm human trial in which participants received dietary supplementation with 1.5 g/day ARA (n = 9) or placebo (n = 10) for a duration of 4-weeks. This dose was based on a recent study which reported apparent ergogenic effects of 1.5 g/day ARA in men participating in resistance training [55]. Additionally, this amount of ARA is typical of that provided in a recommended dose of dietary supplements currently marketed to athletes and body builders [e.g. 55,56]. The duration of intervention was chosen based on prior studies showing that incorporation of dietary n-3 PUFA into plasma and muscle was very rapid and evident between 2 and 4 weeks [57], and on the basis that changes in tissue fatty acid profile and the relation to inflammation (rather than body composition) was our primary outcome. Blood and skeletal muscle biopsy samples were obtained in the morning at rest following an overnight fast, both at baseline and at week 4. Participants were instructed to maintain their habitual diet and exercise training regime throughout the duration of the study, but to schedule their exercise training program and experimental visits so as to refrain from training their legs for ≥72 h prior to each testing session. Participants were instructed to avoid vigorous physical activity of any kind and consumption of alcohol in the preceding 24 h to each laboratory visit. Participants recorded their evening meal on their laboratory visit at baseline and were asked to replicate this evening meal prior to their follow up visit on week 4. Participants consumed their final dose of the allocated dietary supplementation on the evening of day 28 and did not consume an acute dose on the morning of each of the testing session.

2.3. Dietary supplements

The dietary supplement capsules used in this study were provided by DSM Nutritional Products (Heerlen, Netherlands). Supplemental ARA was provided in the form of ARASCO® capsules which contain a mixture of oil extracted from the unicellular fungus *Mortierella alpina* and high oleic sunflower oil. Each 1 g ARASCO® capsule contained ~396 mg ARA according to the manufacturer. The placebo capsules used were indistinguishable from the experimental capsules but contained a soy-corn oil blend (1 g per capsule). The ARA group was provided with an estimated supplemental dose of 1.584 g/day ARA via oral ingestion of 4 × 1 g ARASCO® capsules. The placebo group received 4 g/day of corn-soy oil blend administered as 4 × 1 g placebo capsules. Participants were instructed to consume one dose of 2 × 1 g capsules in the morning and a second dose of two 2 × 1 g capsules in the evening each day for 28 days. On days in which participants undertook resistance exercise training, they were instructed to ingest one of these two doses (~800 mg ARA) 30 min pre-exercise.

2.4. Blood collection and handling

Venous blood samples were collected in a resting state following an overnight fast at baseline and again at week 4. Blood samples were collected from the antecubital vein into BD Vacutainer® K_2 EDTA tubes (BD 367525) for plasma separation and BD Vacutainer Plastic Serum Tubes (Silica) (BD 368975) for serum separation. Plasma tubes were immediately centrifuged at 1500*g* for 15 min at 4 °C. Serum tubes were allowed to clot at room temperature for 15 min prior to immediate centrifugation at 1500*g* for 15 min at 4 °C. Aliquots (~1 mL) of serum and plasma were immediately collected, flash frozen in liquid nitrogen and stored at −80 °C until further analysis.

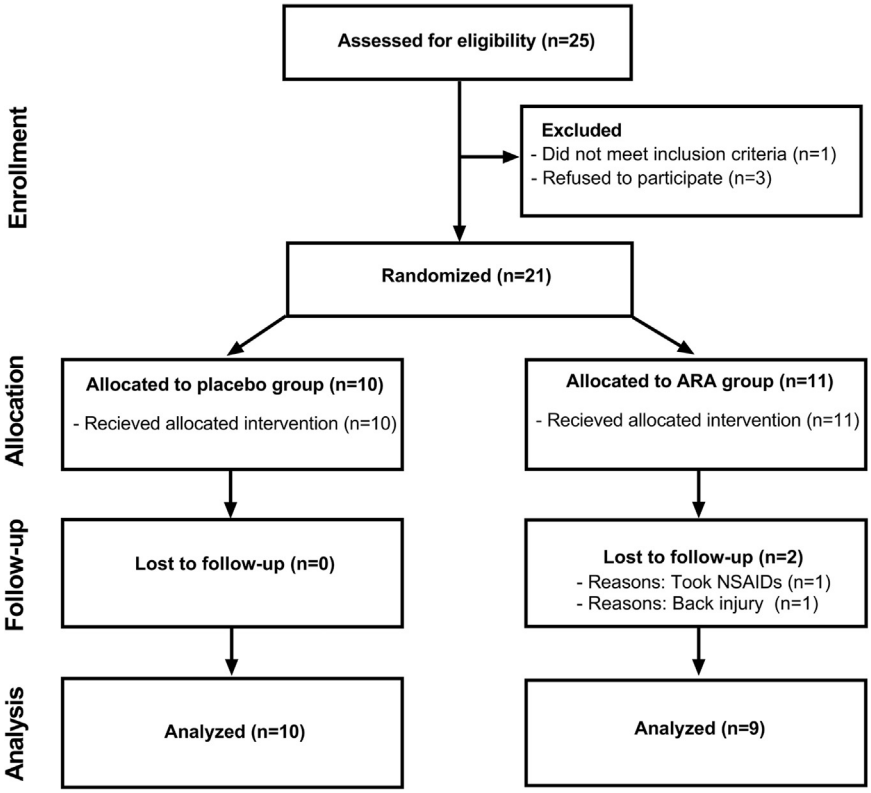

Fig. 1. CONSORT diagram of the randomization and flow of participants through the study.

2.5. Skeletal muscle biopsy sampling

Skeletal muscle biopsy samples were obtained in a resting state following an overnight fast at baseline and week 4. Biopsy samples were taken from the *vastus lateralis* under local anesthesia (xylocaine 1%) by percutaneous needle biopsy technique modified for manual suction using a 5 mm Bergstrom biopsy needle. Muscle biopsies were obtained from the dominant limb at baseline and the contralateral (non-dominant) limb at week 4.

2.6. Fatty acid analysis

Fatty acid composition was analyzed via direct transesterification of the plasma, ARA/placebo capsule oils (six separate oil aliquots per group) and homogenised muscle samples followed by gas chromatography [58]. Methanol: toluene (4:1 v/v) containing C19:0 as internal standard was added to the 200 µl of the plasma, homogenised muscle samples, or approximately 1 mg of oil samples. Acetyl chloride (200 µl) was added dropwise while vortexing and then samples were heated for 1 h at 100 °C. The tubes were cooled to room temperature, 6% K_2CO_3 added and centrifuged (3000*g*, 5 min, 4 °C). The upper toluene phase was collected and stored in a gas chromatograph (GC) vial at −20 °C for GC analysis.

Fatty acid methyl esters were analyzed by gas chromatography (Shimadzu GC- 2010 Plus system) using a 30 m × 0.25 mm ID Restek Famewax (Shimadzu Scientific) fused silica capillary column as previously described [59]. Ultra-pure hydrogen was used as a carrier and the oven temperature was programmed from 130 °C to 225 °C at a rate of 6 °C per min. Injector and detector temperatures were set at 220 °C and 230 °C, respectively. A split ratio of 1:30 and an injection volume of 1 µl was used. A known fatty acid mixture was used to compare to analyzed samples to identify peaks according to retention time. Calibration curves for each fatty acid were built using a range of concentrations of a mixture of authentic fatty acid standards. Chromatography data were recorded and integrated using LabSolutions software (Version 5.81 SP1). Individual fatty acid concentrations were reported as a percentage of total fatty acids measured.

2.7. RNA Extraction from PBMCs and muscle tissue

Whole EDTA blood (2 mL) was layered over 2 mL of Histopaque solution (Sigma-Aldrich, St. Louis, MO, USA) and centrifuged for 30 min at 400 × *g* at room temperature. Peripheral blood mononuclear cells (PBMCs) were aspirated from the upper layer interface, washed twice with phosphate buffered saline and pelleted by centrifugation at 250 × *g* for 10 min at room temperature. Total RNA was extracted from the PBMC cell pellets and approximately 20 mg of frozen muscle tissue using the AllPrep® DNA/RNA/miRNA Universal Kit (QIAGEN GmbH, Hilden, Germany). RNA concentration was measured using a NanoPhotometer® N60 (IMPLEN, Inc., Westlake Village, CA, USA). RNA was reverse transcribed using a High Capacity RNA-to-cDNA Kit (Life Technologies, Carlsbad, CA, USA).

2.8. Real-Time polymerase chain reaction (RT-PCR) analysis

RT-PCR was performed on a LightCycler 480 (Roche Applied Science, Penzberg, Germany) using SYBR™ Green I DNA-binding dye. Samples were run in duplicate 10 µl reaction volumes. Results are expressed as each participant's fold change in mRNA expression from their respective baseline sample following normalization to an endogenous control using the $2^{-\Delta\Delta Ct}$ method. The geometric mean of human *TATA-box binding protein* (*TBP), hypoxanthine phosphoribosyltransferase 1 (HPRT1)* and *valosin containing protein* (*VCP)* mRNA expression was used as an endogenous control. Primers (Table 1) were obtained from Invitrogen (Life Technologies, Carlsbad, CA, USA).

2.9. Serum and whole blood analyses

Serum and whole blood samples were used to evaluate clinical markers. Blood serum samples were assayed for comprehensive

Table 1
RT-PCR primer sequences.

Target	Primer sequence
ITGAM (forward	TCAGGTGGTGAAAGGCAAGG
ITGAM (reverse)	ATCTGTCCTTCTCTTAGCCGA
CEACAM8 (forward)	TGCGGAACGTCACCAGAAAT
CEACAM8 (reverse)	GTCTCCGGATGTACGCTGAA
ELANE (forward)	CGTGGCGAATGTAAACGTCC
ELANE (reverse)	TTTTCGAAGATGCGCTGCAC
CD68 (forward)	GCTACTGGCAGCCCAGG
CD68 (reverse)	CGTGAAGGATGGCAGCAAAG
CD163 (forward)	GCGGCTTGCAGTTTCCTCAA
CD163 (forward)	CTGAAATCAGCTGACTCATGGGA
MRC1 (forward)	CGATCCGACCCTTCCTTGAC
MRC1 (reverse)	TGTCTCCGCTTCATGCCAT
IL1B (forward)	TTCGAGGCACAAGGCACAA
IL1B (reverse)	TGGCTGCTTCAGACACTTGAG
CCL2 (forward)	GCAATCAATGCCCCAGTCAC
CCL2 (reverse)	CTTGAAGATCACAGCTTCTTTGGG
TNF (forward)	AGCCCATGTTGTAGCAAACC
TNF (reverse)	TGAGGTACAGGCCCTCTGAT
IL6 (forward)	TCAATGAGGAGACTTGCCTGG
IL6 (reverse)	GGGTCAGGGGTGGTTATTGC
MYOG (forward)	GGCCAAACTTTTGCAGTGAATATT
MYOG (reverse)	TCGGATGGCAGCTTTACAAACAAC
MYOD (forward)	CGGCATGATGGACTACAGCG
MYOD (reverse)	CAGGCAGTCTAGGCTCGAC
HPRT1 (forward)	CCTGGCGTCGTGATTAGTGAT
HPRT1 (reverse)	TCGAGCAAGACGTTCAGTCC
TBP (forward)	TGTGCTCACCCACCAACAAT
TBP (reverse)	TCTGCTCTGACTTTAGCACCTG
VCP (forward)	AAACTCATGGCGAGGTGGAG
VCP (reverse)	TGTCAAAGCGACCAAATCGC

RT-PCR, real time polymerase chain reaction.

metabolic panels including aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), myoglobin, glucose, insulin, triglycerides (TG), total cholesterol, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol using a Roche C311 autoanalyzer, (Roche, Mannheim, Germany) by enzymatic colorimetric assay. Complete blood cell counts including red cell counts, hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, white blood cell counts, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were analyzed via flow cytometry by LabPLUS (Auckland, New Zealand).

2.10. Dual energy x-ray absorptiometry

Dual energy x-ray absorptiometry (DXA) was used to measure body composition. A whole-body rapid scan DXA (model iDXA, GE-Lunar, Madison, WI) was performed to determine total body fat, fat-free soft tissue and bone mineral content. Lean mass (proxy for skeletal muscle mass) was calculated as the tissue mass that was fat-free and bone free. DXA scans were performed first thing in the morning participant arrival to the laboratory after an overnight fast at both baseline and week 4.

2.11. pqCT

Thigh muscle and lower leg muscle cross sectional area (CSA) was determined by peripheral quantitative computed tomography (pqCT). Three scans were performed in order to measure CSA at (i) mid-thigh level 50% of the distance between the medial edge of the greater trochanter and the intercondyloid fossa (femur length), as well as (ii) proximally at 20% of femur length in order to generate data on CSA which is representative of the entire muscle group. (iii) Lower leg muscle CSA was determined at 66% of tibia length from the lateral malleolus to the lateral knee joint space. The density threshold for muscle was set at 40 mg/cm^3 (contour mode 1/peel mode 1). Cortical area was then subtracted using a threshold of 280 mg/cm^3 (contour mode 1/peel mode 1) to yield muscle CSA. All scans were analyzed by the same operator. pqCT scans were performed in the morning following an overnight fast immediately subsequent to the DXA scan at baseline and week 4.

2.12. Statistical analysis

A priori samples size calculations were conducted using change in muscle n-6 PUFA content as the primary outcome. Based on the change in muscle total n-3 PUFA observed following 4-weeks of n-3 supplementation previously [57], 10 participants per group were required to achieve 90% power. Differences in plasma/muscle fatty acid profile, clinical parameters, body composition, muscle strength and mRNA expression in PBMCs and muscle were assessed with two-way repeated measures ANOVA with time as a within participant factor and group as a between participant factor. Pair-wise differences were assessed with Holm-Sidak post hoc tests. Statistical analysis was performed using SigmaPlot 12.3. All data are reported as mean ± standard error of the mean (SEM) and statistical significance was determined at $p \leq 0.05$.

3. Results

3.1. Participant characteristics

Participant characteristics are displayed in Table 2. There was no difference between groups at baseline for age, height, weight, or muscle strength.

3.2. Capsule fatty acid composition

The fatty acid composition of the placebo and ARA capsules is shown in Table 3. Each placebo capsule contained 683.70 mg total fatty acids composed primarily of linoleic acid (C18:2n-6) (400.73 mg or 58.61%) and oleic acid (C18:1n-9) (139.42 mg or 20.39%). Other minor constitutes included palmitic acid (C16:0) (80.81 mg or 11.82%) and stearic acid (C18:0) (22.25 mg or 3.25%). The placebo oil also contained small amounts of the parent n-3 PUFA alpha-linolenic acid (C18:3n-3) (22.30 mg or 3.26%). Each ARA capsule contained 748.58 mg total fatty acids composed primarily of ARA (C20:4n-6) (373.98 mg or 49.96%) and oleic acid (C18:1n-9) (113.12 mg or 15.11%). Other minor fatty acids included palmitic acid (C16:0) (62.28 mg or 8.32%), stearic acid (C18:0) (58.05 mg or 7.75%) and linoleic acid (C18:2n-6) (56.46 mg or 7.52%). Smaller amounts of gamma-linolenic acid (C18:3n-6) (22.78 mg (3.04%)), dihomo-gamma-linolenic acid (C20:3n-6) (25.04 mg (3.34%)) and eicosadienoic acid (C20:2n-6) (4.82 mg (0.64%)) were also detected in the ARA oil.

3.3. Plasma fatty acid profile

3.3.1. Plasma n-6 PUFA

Plasma fatty acid profiles are displayed in Table 4. Time × group interaction effects were observed for plasma composition of ARA

Table 2
Participant characteristics.

	Placebo (n = 10)		ARA (n = 9)		
	Mean	SEM	Mean	SEM	T-test
Age (years)	24	1.5	26	1.3	p = 0.33
Weight (kg)	91.8	3.32	90.6	4.13	p = 0.81
Height (cm)	181	2.78	180	3.08	p = 0.73
Leg press 1 RM (kg)	225	18.2	253	37.5	p = 0.29
Leg extension 1 RM (kg)	133	7.93	121	7.06	p = 0.34

ARA, arachidonic acid; RM, repetition maximum.

Table 3
Absolute (mg) and relative (%) fatty acid composition of placebo and arachidonic acid (ARA) capsules (1 g). Values are the mean composition of three independent oil samples.

		mg		%	
Fatty acid		Placebo	ARA	Placebo	ARA
C14:0	Myristic acid	0.4	2.0	0.1	0.3
C16:0	Palmitic acid	80.8	62.3	11.8	8.3
C16:1n-7	Palmitoleic acid	1.1	0.5	0.2	0.1
C17:0	Margaric acid	0.6	2.0	0.1	0.3
C18:0	Stearic acid	22.3	58.0	3.3	7.8
C18:1n-9	Oleic acid	139.4	113.1	20.4	15.1
C18:1n-7	Vaccenic acid	8.2	2.5	1.2	0.3
C18:2n-6	Linoleic acid	400.7	56.3	58.6	7.5
C18:3n-6	γ-Linolenic acid	–	22.8	–	3.0
C18:3n-3	α-Linolenic acid	22.3	–	3.3	–
C20:0	Arachidic aicd	2.7	5.4	0.4	0.7
C20:1n-9	Gondoic acid	2.0	2.0	0.3	0.3
C20:2n-6	Eicosadienoic acid	–	4.8	–	0.6
C20:3n-6	Dihomo-γ-linolenic acid	–	25.0	–	3.3
C20:4n-6	Arachidonic acid	–	374.0	–	50.0
C20:5n-3	Eicosapentaenoic acid	–	–	–	–
C22:0	Behenic acid	2.0	10.0	0.3	1.3
C22:5n-3	Docosapentaenoic acid	–	–	–	–
C22:6n-3	Docosahexaenoic acid	–	–	–	–
C24:0	Lignoceric acid	1.2	7.9	0.2	1.1
C24:1n-9	Nervonic acid	–	–	–	–
Total		683.7	748.6	100.0	100.0

ARA, arachidonic acid.
– Denotes fatty acids which were not detected.

($p < 0.001$), linoleic acid ($p = 0.007$), gamma-linolenic acid ($p = 0.026$) and dihomo-gamma-linolenic acid ($p < 0.001$). ARA made up 8.88% of plasma lipids in the placebo group and 8.37% in the ARA group at baseline (between group $p = 0.66$). Plasma ARA increased ~2-fold to 16.24% in the ARA group at week 4 ($p < 0.001$), but remained unchanged at 9.66% in the placebo group ($p = 0.12$). Gamma-linolenic acid composed 3.68% of total plasma lipids in the placebo group and 3.47% in the ARA group at baseline. Gamma-linolenic acid content increased to 5.39% in the ARA group at week 4 ($p = 0.034$), but remained unchanged at 2.77% in the placebo group ($p = 0.28$). Plasma abundance of both ARA ($p < 0.001$) and gamma-linolenic acid ($p = 0.045$) were greater at week 4 in the ARA group compared to placebo. The change in plasma ARA content from baseline at week 4 was higher in the ARA group (+7.9%) compared to placebo (+0.83%) ($p < 0.001$) (Fig. 2A).

Linoleic acid comprised 26.02% of total plasma lipids in the placebo group and 24.84% in the ARA group at baseline. Following 4-weeks of supplementation, linoleic acid decreased to 20.98% in the placebo group ($p < 0.001$) and to 14.36% in the ARA group ($p < 0.001$). Dihomo-gamma-linolenic acid content increased from 1.84% of total plasma fatty acids to 2.09% at week 4 in the placebo group ($p = 0.005$), but decreased from 1.77% to 1.46% at week 4 in the ARA group ($p = 0.002$). Plasma abundance of both linoleic acid and dihomo-gamma-linolenic acid were lower in the ARA group at week 4 ($p < 0.001$).

3.3.2. Plasma n-3 PUFA

There was a time × group interaction effect ($p = 0.044$) for plasma composition of eicosapentaenoic acid. Eicosapentaenoic acid made up 1.07% of plasma fatty acids in the placebo group and 1.37% in the ARA group at baseline (between group $p = 0.10$). Eicosapentaenoic acid decreased to 0.90% in the ARA group at week 4 ($p = 0.009$), but remained unchanged at 1.07% in the placebo group ($p = 0.96$). Nevertheless, there remained no between group difference in plasma content at week 4 ($p = 0.36$). There was no effect of ARA supplementation on plasma content of other n-3 PUFA.

Table 4
Percentage composition of fatty acids in total plasma lipids before (pre) and after (post) 4-weeks of dietary supplementation with placebo or arachidonic acid (ARA).

		Placebo (n = 10)				ARA (n = 9)			
		Pre		Post		Pre		Post	
Fatty acid	Common name	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C14:0	Myristic acid	1.08	0.15	1.11	0.13	1.18	0.21	1.18	0.09
C14:1n-9	Myristoleic acid	–	–	–	–	–	–	–	–
C15:0	Pentadecylic acid	–	–	–	–	–	–	–	–
C16:0	Palmitic acid	23.49	0.60	25.76	0.49*	23.57	0.67	25.40	0.44*
C16:1	Palmitoleic acid	1.65	0.18	1.88	0.16	1.99	0.25	1.83	0.14
C17:0	Margaric acid	0.31	0.02	0.33	0.02	0.35	0.02	0.38	0.03
C18:0	Stearic acid	7.30	0.16	8.46	0.23*	7.48	0.21	8.15	0.24*
C18:1n-9	Oleic acid	14.65	0.66	15.52	0.65	15.10	0.25	14.10	0.64
C18:1n-7	Vaccenic acid	2.57	0.24	2.46	0.11	2.62	0.09	2.92	0.17
C18:2n-6	Linoleic acid	26.02	1.36	20.89	1.26*	24.84	0.89	14.36	0.43*#
C18:3n-6	γ-Linolenic acid	3.68	0.90	2.76	0.82	3.47	0.75	5.39	1.03*#
C18:3n-3	α-Linolenic acid	0.88	0.08	0.93	0.07	0.99	0.11	0.82	0.07
C20:0	Arachidic aicd	0.25	0.01	0.29	0.02	0.30	0.02	0.28	0.01
C20:1n-9	Gondoic acid	0.16	0.03	0.17	0.02	0.15	0.02	0.10	0.01#
C20:2n-6	Eicosadienoic acid	0.17	0.02	0.19	0.02	0.20	0.04	0.12	0.01*
C20:3n-6	Dihomo-γ-linolenic acid	1.84	0.14	2.09	0.15*	1.77	0.10	1.46	0.07*#
C20:4n-6	Arachidonic acid	8.88	0.60	9.66	0.72	8.37	0.62	16.24	1.15*#
C20:5n-3	Eicosapentaenoic acid	1.07	0.11	1.07	0.11	1.37	0.19	0.90	0.10*
C22:0	Behenic acid	0.74	0.02	0.85	0.05	0.81	0.05	0.81	0.02
C22:1n-9	Erucic acid	–	–	–	–	–	–	–	–
C22:5n-3	Docosapentaenoic acid	0.57	0.04	0.60	0.05	0.64	0.03	0.64	0.03
C22:6n-3	Docosahexaenoic acid	2.66	0.13	2.78	0.14	2.80	0.37	2.76	0.26
C24:0	Lignoceric acid	0.75	0.03	0.83	0.03*	0.76	0.05	0.83	0.04*
C24:1n-9	Nervonic acid	1.25	0.06	1.37	0.06	1.23	0.07	1.32	0.08

ARA, arachidonic acid.
Mean values were significantly different from those of pre-supplementation (2-way ANOVA and holm-sidak post hoc test): *$p < 0.05$.
Mean values were significantly different from those of the placebo group (2-way ANOVA and holm-sidak post hoc test): #$p < 0.05$.
– Denotes fatty acids which were not detected.

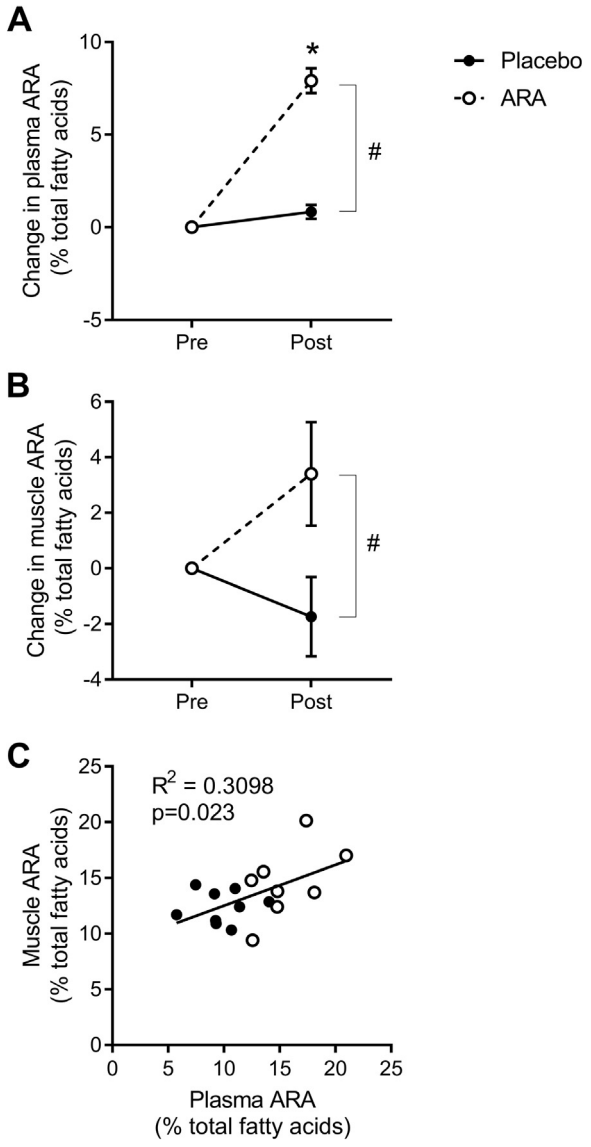

Fig. 2. : *Changes in plasma and skeletal muscle ARA abundance with dietary supplementation.* (A) Absolute change in plasma ARA content (% of total plasma fatty acids) before (Pre) and after (Post) 4-weeks of supplementation with placebo or ARA capsules. (B) Absolute change in skeletal muscle ARA content (% of total muscle fatty acids) before (Pre) an after (Post) 4-weeks of supplementation with placebo or ARA capsules. (C) Relationship between the abundance of ARA in plasma and muscle lipids following 4-weeks of supplementation for participants in the placebo and ARA groups. *$p < 0.05$ vs baseline within group. #$p < 0.05$ between groups post-supplementation. Values are mean ± SEM.

3.3.3. Plasma saturated fatty acids

There were main effects of time, but no effect of group, or group × time interactions for plasma composition of palmitic acid ($p < 0.001$), stearic acid (C18:0) and lignoceric acid (C24:0) ($p = 0.005$). Palmitic acid increased from 23.49% at baseline to 25.76% post-supplementation in the placebo group ($p < 0.001$) and from 23.57% at baseline to 25.40% in the ARA group ($p = 0.002$). Stearic acid increased from 7.30% to 8.46% in the placebo group ($p < 0.001$) and from 7.48% to 8.15% in the ARA group ($p = 0.005$). Lignoceric acid increased from 0.75% to 0.83% in the placebo group ($p = 0.032$) and from 0.76% to 0.83% in the ARA group ($p = 0.045$).

3.4. Skeletal muscle fatty acid profile

3.4.1. n-6 PUFA

Muscle fatty acid profiles are displayed in Table 5. There was time × group interaction effects for muscle abundance of ARA ($p = 0.046$) and dihomo-gamma-linolenic acid ($p = 0.011$). ARA made up 13.97% of muscle lipids in the placebo group and 11.93% in the ARA group at baseline. ARA tended to increase in the ARA group to 14.60% of muscle fatty acids at week 4 ($p = 0.059$), and was unchanged at 12.38% in the placebo group ($p = 0.31$). There was no difference between groups for muscle abundance of ARA before ($p = 0.17$) or after supplementation ($p = 0.22$) (Table 5). Although, the change in muscle ARA from baseline to week 4 was greater in the ARA group (+3.40% of total fatty acids) compared to placebo (−1.76% of total fatty acids) ($p = 0.009$) (Fig. 2B). Muscle ARA content was also positively correlated with plasma ARA abundance at week 4 ($R^2 = 0.31$, $p = 0.023$) (Fig. 2C). Dihomo-gamma-linolenic acid made up 1.50% of muscle lipids in the placebo group and 1.10% in the ARA group at baseline. Dihomo-gamma-linolenic acid content increased to 1.59% in the ARA group at week 4 ($p = 0.003$), but remained unchanged at 1.50% in the placebo group ($p = 0.62$). Dihomo-gamma-linolenic acid was lower in the ARA group compared to placebo at baseline ($p = 0.014$), but no longer differed between groups at week 4 ($p = 0.39$). The change in muscle dihomo-gamma-linolenic acid from baseline was greater in the ARA group (+0.55% of total fatty acids) compared to placebo (−0.08% of total fatty acids) ($p < 0.001$). Linoleic acid content was overall lower in muscle of the ARA group (22.90%) compared to placebo group (25.96%) irrespective of time-point (main effect of group $p = 0.038$). There was no effect of ARA supplementation on muscle composition of n-6 gamma-linolenic acid.

3.4.2. n-3 PUFA

A time × group interaction effect was observed for muscle composition of alpha-linolenic acid ($p = 0.049$). Muscle alpha-linolenic acid was 0.51% in the placebo group and 0.55% in the ARA group at baseline. Alpha-linolenic acid decreased to 0.39% in the ARA group at week 4 ($p = 0.035$), but remained unchanged at 0.55% in the placebo group ($p = 0.48$). There was no between group differences in muscle alpha-linolenic acid content before ($p = 0.64$) or after ($p = 0.093$) supplementation. Nevertheless, the change in alpha-linolenic acid from baseline at week 4 was lower in the ARA group (−0.18% of total fatty acids) compared to the placebo group (+0.06% of total fatty acids) ($p = 0.010$). There was no effect of placebo or ARA supplementation on muscle content of other n-3 PUFAs.

3.4.3. Other fatty acids

Muscle composition of erucic acid (C22:1n-9) showed a main effect of time ($p < 0.001$), but no effect of group, or time × group interaction. Erucic acid decreased from 0.38% to 0.11% in the placebo group ($p = 0.006$) and from 0.40% to 0.20% in the ARA group ($p = 0.030$) at week 4. There was no effect of ARA supplementation on muscle content of any other saturated fatty acids (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0) or monounsaturated fatty acids (C18:1n-9, 20:1n-9, C18:1n-7, C14:1, C16:1).

3.5. Clinical parameters

3.5.1. Hematology

Blood hematology results are presented in Table 6. Group × time interaction effects were observed for blood platelet counts ($p = 0.047$) and blood monocyte counts ($p = 0.049$). Blood platelet counts were 201 × 10^9/L in the placebo group and 233 × 10^9/L in the ARA group at baseline (between group $p = 0.14$). Platelet number decreased in the ARA group to 206 × 10^9/L ($p = 0.005$), but remained unchanged in the placebo group at 199 × 10^9/L ($p = 0.69$), with no difference between groups at week 4 ($p = 0.69$). Monocyte counts were 0.50 × 10^9/L in the placebo group and 0.55 × 10^9/L in the ARA group at baseline (between group $p = 0.55$). There was a trend for monocytes to decrease at week 4 to 0.49 × 10^9/L in the ARA group ($p = 0.056$), but not in the placebo group (0.53 × 10^9/L, $p = 0.62$). There remained no difference in blood monocyte number between groups at week 4 (between group $p = 0.62$).

Table 5
Percentage composition of fatty acids in total skeletal muscle lipids before (pre) and after (post) 4-weeks of dietary supplementation with placebo or arachidonic acid (ARA).

		Placebo (n = 10)				ARA (n = 9)			
		Pre		Post		Pre		Post	
Fatty acid	Common name	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C14:0	Myristic acid	1.27	0.14	1.40	0.15	1.49	0.28	1.43	0.12
C14:1n-9	Myristoleic acid	0.14	0.03	0.16	0.04	0.21	0.06	0.16	0.03
C15:0	Pentadecylic acid	0.17	0.02	0.23	0.04	0.22	0.02	0.22	0.03
C16:0	Palmitic acid	19.49	0.36	20.73	0.67	20.55	0.98	20.56	0.56
C16:1	Palmitoleic acid	1.66	0.41	1.97	0.39	2.61	0.84	1.91	0.35
C17:0	Margaric acid	0.29	0.02	0.27	0.02	0.29	0.02	0.32	0.03
C18:0	Stearic acid	13.00	0.72	12.53	0.56	12.34	1.02	13.83	0.80
C18:1n-9	Oleic acid	13.60	2.11	14.33	1.19	16.13	2.87	12.94	1.21
C18:1n-7	Vaccenic acid	2.56	0.09	2.51	0.07	2.64	0.18	2.33	0.19
C18:2n-6	Linoleic acid	25.72	0.92	26.20	1.07	22.56	1.63	23.25	1.30
C18:3n-6	γ-Linolenic acid	0.12	0.04	0.11	0.02	0.21	0.08	0.27	0.18
C18:3n-3	α-Linolenic acid	0.51	0.09	0.55	0.05	0.55	0.08	0.39	0.05*
C20:0	Arachidic acid	0.12	0.01	0.09	0.01	0.12	0.02	0.12	0.03
C20:1n-9	Gondoic acid	0.28	0.04	0.27	0.04	0.28	0.06	0.27	0.05
C20:2n-6	Eicosadienoic acid	0.11	0.02	0.11	0.01	0.10	0.01	0.11	0.01
C20:3n-6	Dihomo-γ-linolenic acid	1.50	0.11	1.41	0.07	1.10	0.11	1.59	0.17*
C20:4n-6	Arachidonic acid	13.97	1.31	12.38	0.48	11.93	1.85	14.60	1.12
C20:5n-3	Eicosapentaenoic acid	0.62	0.08	0.49	0.08	1.25	0.30	1.03	0.34
C22:0	Behenic acid	0.20	0.03	0.18	0.03	0.20	0.03	0.22	0.02
C22:1n-9	Erucic acid	0.38	0.06	0.11	0.04*	0.40	0.06	0.20	0.04*
C22:5n-3	Docosapentaenoic acid	1.40	0.10	1.30	0.12	1.36	0.17	1.39	0.13
C22:6n-3	Docosahexaenoic acid	2.88	0.29	2.67	0.20	3.46	0.80	2.85	0.34
C24:0	Lignoceric acid	–	–	–	–	–	–	–	–
C24:1n-9	Nervonic acid	–	–	–	–	–	–	–	–

ARA, arachidonic acid.
Mean values were significantly different from those of pre-supplementation (2-way ANOVA and holm-sidak post hoc test): *$p < 0.05$.
– Denotes fatty acids which were not detected.

There was a main effect of time ($p = 0.011$), but no effect of group ($p = 0.060$), or no group × time interaction for mean corpuscular hemoglobin (MCH). Blood MCH tended to decrease slightly at week 4 in both the placebo (30.3 pg/cell to 30.0 pg/cell, $p = 0.075$) and ARA groups (29.0 pg/cell to 28.7 pg/cell, $p = 0.049$). There was a main effect of time, but no effect of group, or group × time interactions for blood lymphocyte number ($p = 0.009$). Blood lymphocytes increased from baseline at week 4 in the placebo group (1.90×10^9/L to 2.33×10^9/L, $p = 0.004$), but not the ARA group (2.48×10^9/L to 2.37×10^9/L, $p = 0.32$). There was no effect of supplementation on blood hemoglobin, red cell count, hematocrit, mean corpuscular volume, or the number of circulating total white cells (leukocytes), immature granulocytes, neutrophils, basophils or eosinophils.

3.5.2. Blood biochemistry

Blood biochemistry results are presented in Table 7. There were no differences over time or between supplement groups for serum total cholesterol, HDL, LDL, triglycerides (TG), glucose, insulin, alanine aminotransferase (ALT) or creatine kinase (CK). A main effect of group was observed for aspartate aminotransferase (AST), with lower serum AST in the ARA group observed compared to the placebo group at baseline ($p = 0.045$), but no difference between groups at week 4 ($p = 0.29$). There was a main effect of time ($p = 0.010$), but no effect of group ($p = 0.36$) or group × interaction ($p = 0.23$) for the ratio of total

Table 6
Complete blood counts before (pre) and after (post) 4-weeks of dietary supplementation with placebo or arachidonic acid (ARA).

	Placebo (n = 10)				ARA (n = 9)			
	Pre		Post		Pre		Post	
Parameter	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Hemoglobin (g/L)	155	1.15	151	4.87	156	2.86	150	2.75
Red cell count 10^{12}/L	5.16	0.0795	5.05	0.151	5.40	0.099	5.22	0.111
Hct	0.45	0.0039	0.44	0.013	0.45	0.0073	0.44	0.0082
MCV (fL)	87	1.2	87	1.2	84	1.1	83	1.2
MCH (pg)	30.3	0.449	30.0	0.537	29.0	0.373	28.7	0.373*
Platelet count (10^9/L)	201	13.4	199	13.0	233	17.3	207	14.8*
Immature granulocytes (10^9/L)	0.01	0.002	0.02	0.004	0.02	0.004	0.02	0.006
White cell count (10^9/L)	5.81	0.525	6.19	0.540	6.16	0.450	5.92	0.446
Neutrophils (10^9/L)	3.07	0.396	3.06	0.359	3.10	0.351	2.77	0.297
Basophils (10^9/L)	0.03	0.005	0.02	0.004	0.02	0.005	0.02	0.008
Eosinophils (10^9/L)	0.31	0.074	0.24	0.054	0.21	0.047	0.20	0.051
Monocytes (10^9/L)	0.50	0.066	0.53	0.068	0.55	0.028	0.49	0.034
Lymphocytes (10^9/L)	1.90	0.113	2.33	0.180*	2.28	0.152	2.43	0.218

ARA, arachidonic acid; Hct, hematocrit; MCV, mean corpuscular volume MCH, mean corpuscular hemoglobin.
Mean values were significantly different from those of pre-supplementation (2-way ANOVA and holm-sidak post hoc test): *$p < 0.05$.

Table 7
Blood serum biochemistry before (pre) and after (post) 4-weeks of dietary supplementation with placebo or arachidonic acid (ARA).

	Placebo (n = 10)				ARA (n = 9)			
	Pre		Post		Pre		Post	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TG (mmol/L)	1.00	0.11	0.88	0.05	1.17	0.14	0.98	0.15
Total Cholesterol (mmol/L)	4.76	0.25	4.52	0.26	4.75	0.37	4.59	0.40
LDL (mmol/L)	3.02	0.25	2.92	0.27	3.14	0.34	3.08	0.35
HDL (mmol/L)	1.53	0.14	1.49	0.13	1.23	0.07	1.27	0.07
Total Cholestrol:HDL ratio	3.37	0.40	3.25	0.37	3.94	0.37	3.66	0.32*
Glucose (mmol/L)	5.29	0.14	5.44	0.09	5.64	0.18	5.57	0.10
Insulin (μIU/mL)	7.09	1.12	8.49	0.80	10.50	1.91	7.28	0.78
ALT (U/L)	26.25	2.28	23.38	1.89	27.34	2.14	24.77	3.65
AST (U/L)	28.71	2.28	27.15	1.26	22.84	1.34[#]	24.32	1.41
CK (U/L)	331.40	76.94	327.00	50.01	199.00	18.06	223.22	35.47

ARA, arachidonic acid; TG, triglycerides; LDL, lower density lipoprotein; HDL, high density lipoprotein.
ALT, alanine aminotransferase; AST, aminotransferase; CK, creatine kinase.
Mean values were significantly different from those of pre-supplementation (2-way ANOVA and holm-sidak post hoc test): *$p < 0.05$.
[#] A main effect of group was observed for aspartate aminotransferase (AST), with lower serum AST in the ARA group observed compared to the placebo group at baseline ($p = 0.045$), but no difference between groups at week 4 ($p = 0.29$).

cholesterol: HDL. Total cholesterol: HDL ratio decreased from 3.94 at baseline to 3.66 at week 4 in the ARA group ($p = 0.022$), but was unchanged with placebo ($p = 0.25$).

3.6. PBMC mRNA expression

A group × time interaction effect was found for PBMC mRNA expression of the granulocyte marker CD66b (*CEACAM8*) ($p = 0.044$) and trend towards a group × time interaction effect was found for the granulocyte marker neutrophil elastase (*ELANE*) ($p = 0.062$). The fold change from baseline at week 4 for both *CEACAM8* ($p = 0.008$) and *ELANE* ($p = 0.015$) was lower in the ARA group (*CD66b* 0.55-fold; *ELANE* 0.46-fold) compared to placebo (*CEACAM8* 1.34-fold; *ELANE* 2.02-fold) (Fig. 3A). There was no effect of ARA on mRNA expression of the general leukocyte marker CD11b *(ITGAM)*, the pan monocyte/macrophage marker CD68 (*CD68*) or the alternative activation macrophage markers CD163 (*CD163*) and CD206 (*MRC1*) (Fig. 3A).

There was a main effect of time ($p = 0.011$), but no effect of group ($p = 0.15$) or group × time interaction ($p = 0.15$) for PBMC mRNA expression of the pro-inflammatory cytokine interleukin 1 beta (*IL1B)*. *IL1B* mRNA increased at week 4 in the placebo group (1.46-fold, $p = 0.013$), but was unchanged in the ARA group (1.14-fold; $p = 0.58$) (Fig. 3B). The fold change in PBMC *IL1B* expression at week 4 also tended to be lower in the ARA group compared to placebo ($p = 0.075$). There was a main effect of time ($p = 0.020$), but no effect of group ($p = 0.89$) or group × time interaction ($p = 0.89$) for PBMC expression of tumor necrosis factor alpha (*TNF*). *TNF* mRNA increased at week 4 overall, but post hoc analysis did not detect a difference from baseline in either group (placebo $p = 0.19$, ARA $p = 0.14$). There was no effect of ARA supplementation on PBMC mRNA expression of interleukin 6 (*IL6)* or monocyte chemoattractant protein-1 (*CCL2)* (Fig. 3B).

3.7. Skeletal muscle mRNA expression

A group × time interaction effect was found for muscle expression of the myogenic regulatory factors MyoD (*MYOD1*) ($p = 0.007$) and myogenin (*MYOG*) ($p = 0.017$). The fold change from baseline for muscle *MYOD* and *MYOG* mRNA at week 4 was greater in the ARA group (*MYOD1* 1.50-fold, *MYOG* 1.34-fold) compared to placebo (*MYOD* 0.56-fold, *MYOG* 0.56-fold) (Fig. 3E). There was no effect of ARA supplementation on muscle mRNA expression on any of the measured immune cell surface markers (*ITGAM*, *ELANE*, *CEACAM8*, *CD68*, *CD163*, *CD206*) (Fig. 3C) or inflammatory cytokines (*IL1B*, *IL6*, *CCL2, TNF*) (Fig. 3D).

3.8. Body composition

3.8.1. DEXA

Participant body composition at baseline and following 4 weeks of ARA supplementation is displayed in Table 8. There was a group × time interaction effect for body mass ($p = 0.013$). Body mass was 92.41 kg in the placebo group at baseline and 91.23 kg in the ARA group (between group $p = 0.82$). Body mass increased to 93.76 kg in the placebo group at week 4 ($p = 0.006$), but remained unchanged at 90.85 kg with ARA ($p = 0.41$). While there was no between group difference in absolute body mass at week 4 (between group $p = 0.59$), the change in body mass from baseline was lower in the ARA group (−0.37 kg or −0.33%) compared to placebo (+1.35 kg or +1.4%) ($p < 0.001$).

There was a trend for a group × time interaction effect for fat mass ($p = 0.054$) and a group × time interaction effect for percentage change in fat mass ($p = 0.042$). Fat mass was 16.59 kg in the placebo group and 17.50 kg in the ARA group at baseline (between group $p = 0.73$) and did not change at week 4 in the placebo group (17.08 kg; $p = 0.090$) or the ARA group (17.17 kg; $p = 0.27$). While there remained no difference between groups for fat mass at week 4 (between group $p = 0.97$), the change in fat mass from baseline was lower in the ARA group (−0.33 kg or −1.7%) compared to placebo (+0.49 kg or +3.8%) ($p = 0.004$). There was no effect of ARA supplementation on lean body mass, bone mineral content or relative body composition (% fat mass, % lean mass).

3.8.2. Muscle volume (pQCT)

There were main effects of time, but no main effect of group or group × time interaction effects for muscle CSA of the lower leg ($p = 0.049$) and 20% of femur length ($p = 0.043$). Additionally, a statistical trend for a main effect of time was observed for muscle CSA at 50% of femur length ($p = 0.086$). Muscle CSA the lower leg increased at week 4 in the placebo group ($p = 0.022$), but not the ARA group ($p = 0.64$). Muscle CSA at 20% of femur length increased from baseline overall at week 4, but post hoc comparisons did not show a significant change from baseline length for the placebo group ($p = 0.071$) or the ARA group ($p = 0.257$) Table 9.

4. Discussion

The present study investigated the effect of 4-weeks of dietary supplementation with 1.5 g/day ARA on fatty acid profile and inflammation in resistance exercise trained men. This manuscript reports

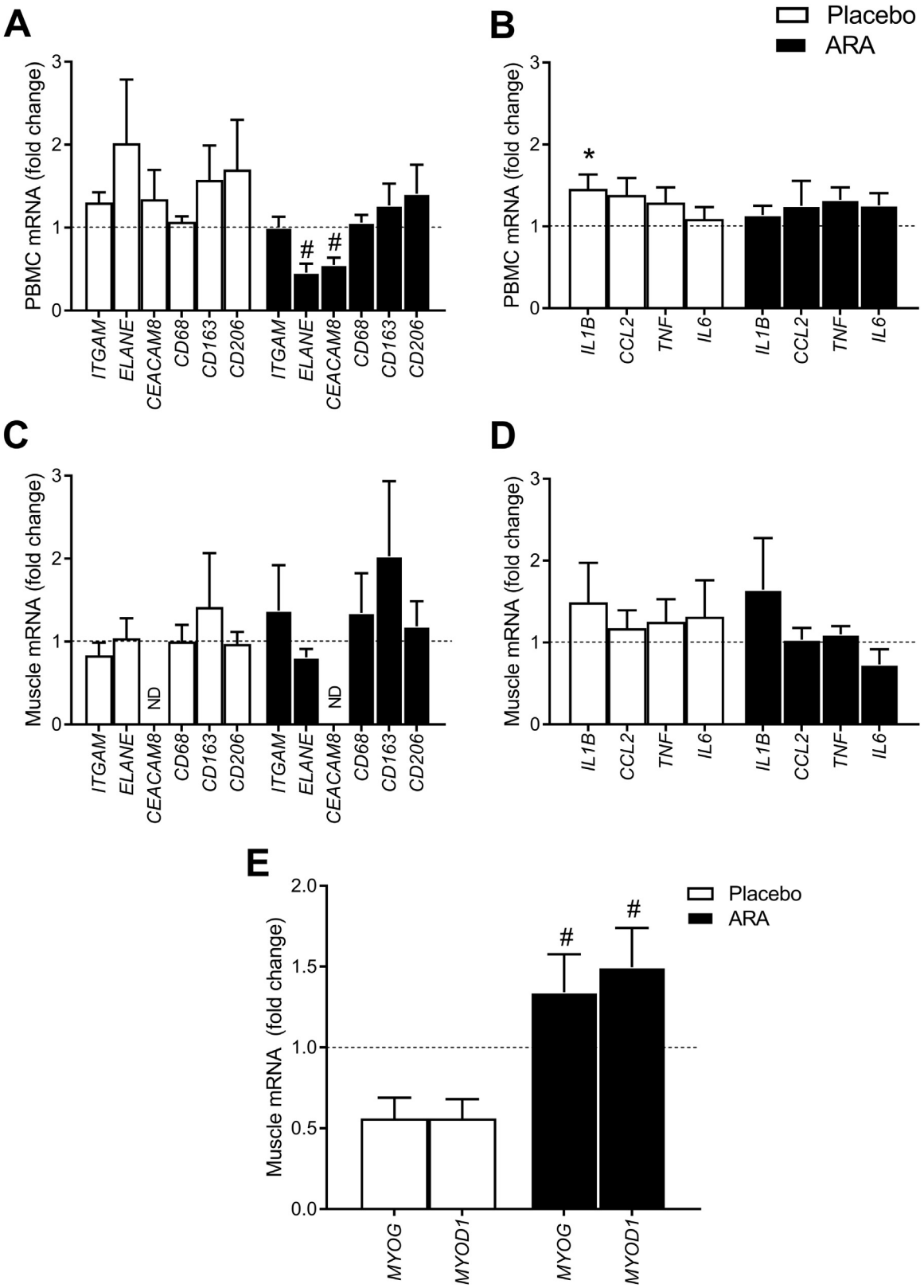

Fig. 3. *: Skeletal muscle and PBMC mRNA expression:* mRNA expression of immune cell markers in PBMCs (A), inflammatory cytokines in PBMCs (B), immune cell markers in muscle (C), inflammatory cytokines in muscle (D) and myogenic regulatory factors in muscle (E). ND denotes mRNA levels not reliably detected. Values are the mean ± SEM of participants' fold change in mRNA expression from respective baseline levels. *$p < 0.05$ vs baseline within group. #$p < 0.05$ between groups post-supplementation.

Table 8
Dual-energy x-ray absorptiometry (DXA) derived body composition before (pre) and after (post) 4-weeks of dietary supplementation with placebo or arachidonic acid (ARA).

	Placebo (n = 10)						ARA (n = 9)					
	Pre		Post		% change		Pre		Post		% change	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Total body mass (kg)	92.41	3.41	93.76	3.51*	1.4	0.40*	91.23	4.12	90.85	3.87	−0.33	0.51#
Fat mass (kg)	16.59	1.99	17.08	1.97	3.8	2.0*	17.50	1.75	17.17	1.66	−1.7	1.3#
Lean mass (kg)	71.96	3.12	72.79	3.35	1.0	0.59	70.06	3.06	70.01	2.99	−0.039	0.34
Bone mass (kg)	3.86	0.18	3.89	0.17	0.67	0.50	3.66	0.16	3.66	0.15	0.20	0.40
Fat mass (%)	17.98	2.03	18.32	2.05			19.02	1.48	18.77	1.48		
Lean mass (%)	77.84	1.96	77.53	1.99			76.96	1.46	77.19	1.48		
Bone mass (%)	4.18	0.14	4.15	0.14			4.02	0.08	4.04	0.07		

ARA, arachidonic acid.
Mean values were significantly different from those of pre-supplementation (2-way ANOVA and holm-sidak post hoc test): *$p < 0.05$.
Mean values were significantly different from those of the placebo group (2-way ANOVA and holm-sidak post hoc test): #$p < 0.05$.

Table 9
pQCT determination of muscle volume before (Pre) and after 4-weeks (Post) of dietary supplementation with placebo or arachidonic acid (ARA).

	Placebo (n = 10)				ARA (n = 9)			
	Pre		Post		Pre		Post	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Lower leg								
Non-fat area (cm^2)	96.09	4.13	100.27	4.31*	96.95	4.94	97.71	4.82
Bone area (cm^2)	11.00	0.96	9.72	0.68	8.85	0.46	8.59	0.43
Muscle area (cm^2)	85.09	3.95	90.55	3.97*	88.10	4.79	89.13	4.64
Upper leg (20%)								
Non-fat area (cm^2)	127.90	6.46	131.29	5.77	113.22	4.85	114.74	4.81
Bone area (cm^2)	10.97	0.42	10.91	0.41	10.58	0.56	10.01	0.87
Muscle area (cm^2)	116.93	6.41	120.38	5.64	102.64	4.55	104.73	4.87
Upper leg (50%)								
Non-fat area (cm^2)	219.79	8.79	222.07	8.67	206.32	7.04	207.57	6.76
Bone area (cm^2)	7.43	0.31	7.35	0.28	7.03	0.37	7.05	0.31
Muscle area (cm^2)	212.36	8.68	214.72	8.56	199.29	6.85	200.52	6.60

ARA, arachidonic acid; pQCT, peripheral quantitative computed tomography.
Mean values were significantly different from those of pre-supplementation (2-way ANOVA and holm-sidak post hoc test): *$p < 0.05$.

on the fatty acid profile data and demonstrates that ARA supplementation had clear modulatory effects on the composition of both plasma and muscle lipids. There was also no evidence of heightened basal systemic or intramuscular inflammation in these young male participants who were regularly participating in resistance exercise training. These findings do not support the common assertion that heightened dietary intake and tissue abundance of ARA will promote chronic inflammation in otherwise healthy individuals.

Previous human clinical trials in sedentary populations found that ARA supplementation had little or no effect on blood leukocyte number [36,38,39,44,61] or circulating inflammatory markers [36,38,61]. Nevertheless, many still consider that an excess consumption of ARA is pro-inflammatory. Importantly, ARA and its metabolites are highly transient molecules which act as autocrine/paracrine lipid mediators to influence local inflammation and thus may not be expected to modulate systemic inflammatory markers. Therefore, it was important to assess the effect of ARA on inflammatory responses at the cellular and tissue level. We found no evidence that supplementation with 1.5 g/day ARA influenced resting inflammatory gene expression in PBMCs (monocytes/lymphocytes) or locally within skeletal muscle tissue. Blood monocyte counts, as well as PBMC mRNA expression of neutrophil elastase (*ELANE*) and CD66b (*CEACAM8*), were actually modestly reduced at week 4 in the ARA group in the current study. Similarly, serum levels of the proinflammatory cytokine interleukin-6 were previously reported to be reduced in resistance training men who consumed 1 g/day ARA for 25 days [56]. These changes would appear to reflect, if anything, a reduced basal systemic inflammatory response in men participating in resistance exercise training. In cross sectional studies, individuals with higher plasma ARA have also been found to exhibit lower levels of circulating proinflammatory cytokines [45]. The dose of 1.5 g/day ARA used here was based on a recent study which reported apparent ergogenic effects of this dose of ARA in men participating in a resistance exercise training program [55]. This dose is ~10 fold greater than typical dietary intakes of ARA in the western diet [11,12] and would be potentially difficult to achieve from whole food sources [3]. Nevertheless, it is possible that extreme doses of ARA, such as those that have been tested in animal studies (e.g. 110 mg/kg/day [62] or ~10 g/day for a 90 kg man) may have differing effects on muscle inflammation. Overall these findings do not appear to support the common assertion that an increased dietary intake of ARA (at least up to ~1.5 g/day) will promote chronic inflammation in otherwise healthy individuals. Whether elevated tissue ARA content influences acute phase inflammatory responses which occur during the hours to days following an acute exercise bout, however, remains to be determined [63].

Addition of 1.5 g/day supplemental ARA for 4-weeks approximately doubled plasma lipid ARA content, consistent with previous studies showing that serum/plasma lipid composition is highly sensitive to dietary ARA intake [16,17,36,41–44,64–68]. Since ARA is predominantly incorporated into blood phospholipids [42,64,66], changes in plasma ARA content primarily reflect phospholipid enrichment [40]. In rodents, dietary ARA is also rapidly incorporated into phospholipids of various tissues [13,14,69–73], including skeletal muscle [62]. In contrast, human blood platelets [41] and adipose tissue [40] appear relatively resistant to changes in fatty acid profile with increased dietary ARA intake. We found a significant group × time interaction effect for muscle ARA content, but post hoc analysis was unable to detect any differences between groups for absolute muscle ARA abundance. Nevertheless, the change in muscle ARA content from baseline at week 4 was greater in the ARA group compared to placebo. Therefore, we conclude that dietary ARA was incorporated in muscle tissue, but this effect was more modest and variable than changes observed in plasma.

To our knowledge this is the first study to investigate the effect of n-6 PUFA supplementation on the lipid profile of human muscle tissue. A prior study that provided participants with 5 g/day of n-3 PUFA rich fish oil containing 3.5 g/day eicosapentanoic acid (20:5n-3) and 0.9 g/

day docosahexanoic acid (22:6n-3) for 4-weeks reported a ~2-fold increase in muscle n-3 PUFA content, mainly attributable to incorporation of 20:5n-3 into muscle lipids [57]. We provided a similar 4/g dose of n-6 PUFA rich ARASCO oil which contained ~1.5 g/day ARA; less than the amount of 20:5n-3, but greater than the amount of 22:6n-3 tested in the above study. It is possible that supplementation with higher doses of ARA or longer periods of ARA supplementation may result in greater incorporation of ARA into muscle phospholipids. Importantly however, ingestion of different individual PUFA at the same absolute doses can have highly divergent effects [74,75]. Therefore, doses of n-3 and n-6 PUFA may not be directly comparable between studies.

The ARASCO® oil used here (~50% ARA; Table 3) was chosen to be representative of ARA enriched triglyceride oils extracted from *Mortierella alpina* that are currently manufactured for human consumption [76] and commercially available supplements marketed to athletes and bodybuilders [55,56]. The placebo and ARA oils were well matched for saturated fatty acids and oleic acid and both contained an inconsequentially low amounts of linoleic acid when compared to habitual intakes of ~14.8 g/d [4]. In addition to ARA, the abundance of gamma-linolenic-acid in plasma and dihomo-gamma-linolenic acid in muscle tissue increased post-supplementation. Habitual dietary intake of gamma-linolenic-acid is typically negligible (~5 mg) [44]. Therefore, the small amount of gamma-linolenic-acid present in the ARA oil used here (22.8 mg per capsule or ~90 mg/day) likely contributed to this increase in plasma abundance. Contrary to ARA, gamma-linolenic-acid is predominantly incorporated into neutral plasma lipids including triglycerides and cholesterol esters [77,78]. This may potentially explain why prior studies have not observed any change in gamma-linolenic-acid content of plasma phospholipids in response to supplementation with similar ARA enriched oils [44]. The oil used here also contained small amounts of dihomo-gamma-linolenic acid (25.0 mg per capsule or 100 mg/day) which may have contributed to the increase in muscle abundance of this fatty acid [79]. Both gamma-linolenic acid and dihomo-gamma-linolenic acid have purported anti-inflammatory effects [80]. Therefore, it is possible that some potential pro-inflammatory effects of ARA may have been counteracted by parallel increases in gamma-linolenic acid and dihomo-gamma-linolenic acid intake. Additionally, it is possible that some observed effects on muscle including heightened myogenic gene expression in the ARA group may be mechanistically related to an increase in muscle content of dihomo-gamma-linolenic acid, or decreased in muscle alpha-linolenic acid, rather changes in the muscle abundance of ARA itself.

Incorporation of the n-6 PUFA ARA and gamma-linolenic acid into plasma lipids was accompanied by a large reduction in the plasma abundance of linoleic acid (−42.2%), eicosapentaenoic acid (−34.4%), and dihomo-gamma-linolenic acid (−17.8%). This reduction in linoleic acid is consistent with previous studies testing ≥500 mg/day ARA [36,40,42,44,66,67], whereas smaller doses (80–240 mg/day) have not had this effect [36,43,64,65]. In Japanese participants consuming diets rich in n-3 PUFA (~0.5–1 g/day) ARA supplementation has been found to have no effect on plasma eicosapentaenoic acid [36,42,43,64]. In western populations, however, with a similar plasma eicosapentaenoic acid content to the present study (~1.0–1.5%), reduced plasma eicosapentaenoic acid content has also reported in prior studies [44,67]. In contrast to our results, most prior studies failed to find an effect of ARA supplementation on plasma dihomo-gamma-linolenic acid [40,61,65,68]. Differences between studies including the trained status of our participants, the plasma fraction measured and/or lipid composition of the ARA supplement used may potentially account for these differences.

There was no obvious effect of ARA supplementation on several cardiovascular disease risk factors, which is consistent with prior clinical trials in sedentary individuals [36–44]. In one early study, supplementation with a substantially greater dose of ARA (6 g/day) for 3 weeks was found to increase in-vitro platelet aggregation [66]. Although platelet aggregation was not measured here, comparable doses ARA had no effect on thrombosis in healthy adult males consuming a typical western diet [17,41] or Japanese men consuming a fish based diet [42]. Blood platelet number decreased following supplementation in the ARA group in the current study but remained well within the normal physiological range in both groups. While longer term human clinical trials are required, in support of an apparent lack of a negative effect of ARA supplementation on cardiovascular risk factors, a recent systematic review and meta-analysis of 17 human observational studies found that higher blood ARA content was independently associated with a reduced rather than increased cardiovascular disease risk [81].

Compared to placebo, the ARA group displayed relatively lower gain in fat mass, but no differences in lean mass over the 4-week supplementation period. Prior studies have in general found no apparent effect of ARA supplementation on body composition in sedentary individuals [36,42]. On the other hand, supplementation with 1.5 g/day ARA for 8 weeks was recently reported to promote increases in lean body mass, with no effect on fat mass, in men participating in a resistance exercise training program [55]. The shorter duration of our study and lack of standardization of participant's resistance exercise training regimen may potentially account for the lack of an effect on lean body mass in the current study. It is important to note that body composition was not intended as a primary outcome of this study and as such we did not attempt to standardize participants diet and/or physical activity levels. We are therefore unable to rule out whether the changes in body composition observed here may have resulted from other changes in lifestyle changes that were not assessed.

There are several limitations to this study. The population examined were healthy, young males (18–35 years), who may likely be less impacted by chronic inflammatory stress. This population was selected to represent athletes and bodybuilders who are likely to be potential consumers of such supplements [55,56]. It cannot be concluded from our results that ARA supplementation will not increase inflammatory and/or cardiovascular risk markers in settings of aging or metabolic/inflammatory disease. Our findings do, however, support previous studies showing evidence of apparent physiological benefit and lack of obvious harm of dietary ARA supplementation in sedentary middle aged adults [44,61], the elderly [36,43,65,82] and cirrhosis patients [68]. Thus, future studies investigating the effects of ARA supplementation in settings of skeletal muscle wasting (e.g. aging) may be warranted. A further limitation is that our study was powered to assess changes in tissue fatty acid composition and associated changes in inflammatory makers as primary outcomes. We therefore cannot discount the possibility that this study may be underpowered to detect changes in body composition and cardiovascular risk markers. Finally, we acknowledge that other minor differences in the fatty acid composition of the placebo and ARA supplements, independent of ARA content, may have contributed to some of the results observed.

In conclusion, increasing ARA consumption by 1.5 g/day ARA for 4-weeks in healthy young men participating in resistance exercise training promotes greater changes in plasma and muscle lipid ARA abundance compared to placebo, but this is not associated with any evidence of a heightened systemic or intramuscular basal inflammation. ARA supplementation also had no obvious negative impact on a range of clinical parameters in this population. Therefore, short term increases in dietary ARA intake at least up to 1.5 g/day do not influence local inflammation in healthy young men participating in regular resistance exercise training.

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Conflict of interest

None.

Author contributions

J.F.M, C.J.M, A.J.S and D.C.S designed the study. J.F.M, C.J.M, R.F.D, B.R.D and S.M.M conducted the human clinical trial. J.F.M, S.M.M, R.F.D, K.M.M.A, A.H.C.C and M.G performed sample analysis. J.F.M, C.J.M, A.J.S and D.C.S wrote the manuscript. All authors read and approved the final manuscript.

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