A SYSTEMATIC REVIEW OF THE EFFECTS OF INCREASING ARACHIDONIC ACID INTAKE ON PUFA STATUS, METABOLISM AND HEALTH-RELATED OUTCOMES IN HUMANS

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Abbreviations used: ADP, adenosine diphosphate; ARA, arachidonic acid; CE, cholesteryl ester; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; LC-PUFA, long chain polyunsaturated fatty acid; PG, prostaglandin; PL, phospholipid; RBC, red blood cell, RCT, randomised controlled trial; TG, triglyceride.

Abstract

We conducted a systematic review of randomised controlled trials (RCTs) of increased intake of arachidonic acid (ARA) on fatty acid status and health outcomes in humans. We identified 22 articles from 14 RCTs. Most studies were conducted in adults. These used between 80 and 2000 mg ARA/day and were of 1 to 12 weeks duration. Supplementation with ARA doses as low as 80 mg/d increased the content of ARA in different blood fractions. Overall there seem to be few marked benefits for adults of increasing ARA intake from the typical usual intake of 100-200 mg/day to as much as 1000 mg/d; the few studies using higher doses (1500 or 2000 mg/day) also report little benefit. However, there may be an impact of ARA on cognitive and muscle function which could be particularly relevant in the ageing population. The studies reviewed suggest no adverse effects in adults of increased ARA intake up to at least 1000 to 1500 mg/d on blood lipids, platelet aggregation and blood clotting, immune function, inflammation, or urinary excretion of ARA metabolites. However, in many areas there are insufficient studies to make firm conclusions, and higher intakes of ARA are deserving of further study. Based on the RCTs reviewed, there are not enough data to make any recommendations for specific health effects of ARA intake.

Introduction

Arachidonic acid (ARA) is the common name for all-*cis*-5,8,11,14-eicosatetraenoic acid (Figure 1), commonly abbreviated to 20:4ω-6 or 20:4n-6. ARA is a long chain polyunsaturated fatty acid (LC-PUFA) within the omega-6 (n-6) family. ARA is commonly found in cell membranes, particularly in animals. Therefore meat, especially red meat, but also white meat and fish; offal (organ meat) like liver, kidney and brain; and eggs are sources of ARA. A recent study reported estimated dietary intakes for ARA among adults in 47 developed and 128 developing countries [(1)]; the study reported that 48% of the 175 countries have an ARA intake of < 150 mg/day. Amongst developed countries, mean daily ARA intakes were estimated to be between 100 and 350 mg and ARA contributed < 0.1% of total daily energy intake. Data on ARA intake in specific population subgroups are extremely limited [(2)]. Human milk also contains ARA. Brenna et al. [(3)] reported human milk ARA (as % of total fatty acids (FAs)) from 106 studies in the range of 0.24 to 1% with a median (and SD) of 0.47 $\pm$ 0.13. Many infant formulas are supplemented with ARA at a level of 0.35 to 0.7% of total FAs.

ARA can be synthesised from the essential n-6 PUFA linoleic acid (18:2n-6, LA) by a series of desaturation and elongation reactions as shown in Figure 2. Based upon a study with deuterium-labelled LA, Emken et al. [(4)] reported that 1 to 2.2% of LA is converted to other n-6 PUFAs in healthy young adult males with about 0.5% appearing as ARA.

Despite its relatively low intake from the diet, ARA makes an important contribution to FAs within circulating lipids and in most cells and tissues, particularly to membrane phospholipids (PLs). For example, ARA has been reported to contribute an average of 9.6, 6.6, 15.5, 9.5 and 16% of total FAs in plasma phosphatidylcholine, plasma cholesteryl esters (CEs), red blood cells (RBCs), platelets and blood mononuclear cells in healthy British adults who were non oily-fish consumers and were consuming typical diets [(5)]. The relatively high content of ARA in these pools, in comparison to its intake from the diet, suggests that synthesis from LA is an important pathway and that metabolic mechanisms might serve to concentrate and retain ARA in cell membranes. ARA has a structural role in the brain [(6, 7)], free ARA has roles in cell signalling, and ARA-containing PLs also have roles in cell signalling and are precursors to endocannabinoids that have a range of biological properties [(8)]. A major role of cell membrane ARA is as a substrate for the synthesis of eicosanoids,

which include prostaglandins (PGs), thromboxanes, and leukotrienes. These are formed by metabolism of ARA by cyclooxygenase, lipoxygenase and cytochrome P450 pathways [9-11]. The resulting metabolites have many roles in inflammation and pain, regulation of the immune response, bone turnover, platelet aggregation and blood clotting, smooth muscle contraction, and renal function [9-11]. These well-known functions of ARA-derived metabolites suggest that ARA itself might impact a range of outcomes related to human health. This has been explored in a small number of human studies, frequently using supplements containing ARA. The first such study was by Seyberth et al. [12]. This was a small study involving four healthy men given 6 g of ethyl arachidonate daily for a period of 2-3 weeks. The authors identified an increased content of ARA in plasma triglycerides (TGs), PLs, and CEs and in platelets, and a mean 47% increase in excretion of the major urinary metabolite of ARA. The threshold of adenosine diphosphate (ADP) required to induce platelet aggregation was decreased in all four subjects (by 40 to 90%) which was interpreted as an enhanced potential for blood clotting. This was considered to be a major health concern and most likely explains why there was no further interest in studies with ARA supplements in humans until the mid-1990s [13-17].

The aim of this systematic review is to assess the effects of increasing ARA intake through foods or supplements on ARA levels in different compartments, metabolism and health-related outcomes in humans. All health-related outcomes that have been addressed in randomised controlled trials (RCTs) investigating increased ARA intake through foods or supplementation were considered. Research gaps were identified and we assessed whether recommendations for ARA intake can be made for specific health effects.

Methods

Details of the methods used for the systematic review are published in the PROSPERO international prospective register of systematic reviews (registration number: CRD42017076493).

Criteria for considering studies in this review

Types of study: RCTs in humans were included if they measured any human health-related outcome. Observational and non-randomised interventional studies were excluded.

Types of intervention: Studies were included if they increased intake of ARA through a food or supplement and had a control group with either a lower ARA intake or no ARA

supplementation. We excluded studies assessing the effect of the combination of docosahexaenoic acid (DHA) and ARA or the combination of any other fatty acid with ARA.

Types of population: Studies of human subjects (infants, children and adults) conducted in any country were included, without restriction on age or health status. We excluded animal and *in vitro* studies.

Minimum duration of intervention: Duration of ARA intervention and follow-up varied depending on the study design and health outcome measured; we did not place a restriction on this.

Types of outcome measure: All types of health outcomes, including risk markers of disease were included.

Date of publication: There was no restriction on study inclusion according to year of publication.

Search methods for identification of studies

The Cochrane Central Register of Controlled Trials database was searched on 23 August 2017 and again on 23 March 2018 in an update search, using text words with appropriate truncation and relevant indexing terms (Table 1). The keywords for the search were different synonyms related to the intervention (i.e. arachidonic acid). The systematic review software Covidence (www.covidence.org) was used to facilitate screening of the literature. Titles and/or abstracts of studies retrieved using the search strategy were screened independently by two review authors (a combination of SL, AS, RPM, MF, BS) to identify studies that met the inclusion criteria. The full texts of the potentially eligible studies were retrieved and independently assessed for eligibility by two review team members (a combination of SL, AS, RPM, BS) with final agreement by all authors. Any disagreement between them over the eligibility of particular studies was resolved through discussion between the two relevant reviewers or the whole group. A standardised, pre-piloted form was used to extract data from the included studies for assessment of study quality and evidence synthesis. Extracted information included study setting; study population and participant demographics and baseline characteristics; details of the intervention and control conditions; study methodology; recruitment and study completion rates; outcomes and times of measurement; funding. One of the authors (BvdH, RPM, MF or BS) extracted data and the data were double-checked by another member in the team. A flow chart of the literature identification can be found in Figure 3.

Reference lists of all eligible papers and relevant systematic reviews were searched for additional studies.

Risk of Bias Assessment

Three authors independently (RPM, MF and BS) assessed the risk of bias in included studies by following the Cochrane Risk of Bias guidelines [19] and any disagreement between them was resolved through discussion.

Results

CENTRAL search

2104 titles and abstracts were identified via the electronic search from which 1255 duplicates were removed. Additional references (n=69) were found via reference screening of review papers found in the electronic search, of which 36 were duplicates. In total, 1964 titles were excluded as irrelevant based on title and abstract considering the inclusion and exclusion criteria. The remaining 48 papers were screened based on full text, and 22 were considered as relevant for review inclusion. A flow chart of the literature identification process can be found in Figure 3. The 22 articles included in the systematic review [13-17, 20-36] came from 14 individual studies. Table 2 summarises these studies. Most studies were conducted in healthy young or older adults and several were restricted to men. One study was conducted in breastfeeding women, one in patients with liver cirrhosis, and two in children with parasitic worm infestation. Studies in adults used between 80 and 2000 mg ARA/day, were of 1 to 8 weeks duration and usually used ARA as a supplement

Risk of bias assessment

The overall risk of bias analysis shows that for many studies bias is unclear due to insufficient reporting of required details essential for an informed decision (Figure 4).

Fatty acid composition in different compartments

A number of included studies reported the effect of increased intake of ARA on the FA composition of one or more blood pools.

Nelson and colleagues [13-17] performed a placebo-controlled, random order, cross-over study in 12 healthy male participants housed in a metabolic unit. Participants consumed a

stabilisation diet that contained ~ 200 mg/day ARA for 15 days, and then either continued on the stabilisation diet for 50 days or consumed a diet that provided 1.7 g/day ARA for 50 days. After that, the participants crossed over to the other diet for 50 days, followed by 15 days of the stabilisation diet. Ten participants completed the study. The FA compositions of plasma, plasma TGs, CEs, PLs and free fatty acids, RBCs, adipose tissue [14] and platelets [13] were reported. There was a near doubling of plasma ARA when ARA was consumed (from ~8 to ~16% of total FAs) which was accompanied by a decrease in LA. ARA increased in plasma TGs, CEs (from ~7.5 to ~15% of FAs) and PLs (from ~10% to ~19% of FAs) again mainly at the expense of LA. There was a small increase in ARA in RBCs and platelets but no change in adipose tissue ARA. Increases in ARA content were not reflected in any changes in eicosapentaenoic acid (EPA) or DHA, except in RBCs where DHA content decreased.

Thies et al. [34-36] performed a placebo-controlled, randomised, parallel study in healthy older British subjects: 8 participants received a blend of palm and sunflower seed oils as control while 8 received ~700 mg ARA (from ARASCO) daily for 12 weeks. Participant's habitual intake of ARA was about 150 mg/day. Blood samples were collected at baseline and at 4, 8 and 12 weeks supplementation and then after 4 weeks washout. The FA compositions of plasma PLs [34] and blood mononuclear cells [35] were reported. In plasma PLs, ARA increased from 9.3 to 16% of FAs by 4 weeks and did not increase further, but returned to baseline levels after 4 weeks washout [34]. Plasma PL ARA levels were significantly higher in the ARA group than in the control group at weeks 4, 8 and 12. The increase in ARA in plasma PLs did not significantly affect LA, EPA and DHA levels. In mononuclear cells, ARA increased significantly from ~20% of total FAs at baseline to ~23% at 8 and 12 weeks [35]. This increase was mainly compensated for by a decrease in LA. EPA and DHA levels in mononuclear cells were not affected by ARA supplementation. ARA levels in mononuclear cells were not significantly different between the ARA and control groups.

Pantaleo et al. [29] evaluated if ARA supplementation could increase ARA levels in plasma and RBC lipids in Italian patients with liver cirrhosis. Patients received either 2 g ARA (as 4 g ARASCO) daily or oleic acid as control for 8 weeks. ARA supplementation for 8 weeks significantly increased ARA levels in plasma total lipids, plasma PLs and RBCs. This increase was transitory, since ARA levels returned to baseline 4 weeks after cessation of supplementation. No changes in ARA levels were observed in the control group. There was no significant effect of ARA on LA in plasma total lipids, plasma PLs or RBCs, although LA

was numerically lower in all three pools after ARA compared to before. No results were given for n-3 FA levels, and no between group comparison was made.

Schubert et al. [31] compared the effect of two different fat blends on the FA status of 30 healthy German adults. Participants were randomly distributed into 2 groups receiving for 2 weeks either an oil blend providing 240 mg/day of alpha-linolenic acid, 120 mg/day of EPA, 49 mg/day of stearidonic acid and 73 mg/day of gamma-linolenic acid in three capsules/day or an oil providing 40 mg ARA/day in one capsule/day plus two olive oil capsules/day. Results showed that 40 mg/d ARA supplementation for 2 weeks did not increase plasma or RBC ARA. This lack of change is most likely because of the low amount of ARA provided. Between group comparison, done at each time point, showed that EPA was higher in plasma lipids after the 2 weeks supplementation period in the group receiving the blend containing EPA than in the ARA group.

Kusumoto et al. [26] carried out a double-blind, placebo-controlled study in healthy Japanese men consuming a high-fish diet. One group of men received capsules providing 838 mg ARA incorporated into a TG derived from *Mortierella alpina* (SUNTGA40S) daily for 4 weeks, while another group received capsules with olive oil as control. FAs in serum PLs and TGs were measured in fasted blood at four time points: baseline, after 2 and 4 weeks of supplementation and 4 weeks after the end of supplementation. Supplementation with ARA increased ARA content of serum PLs from 9.6 to 13.7 (after 2 weeks) and 13.9% (after 4 weeks) of total FAs, which was significantly different from baseline, and then ARA content decreased to a level close to that of baseline 4 weeks after the end of supplementation. Serum PL ARA did not change in the control group. The same pattern was observed in serum TGs, although levels of ARA were lower in this lipid class than in PLs. Between group comparison showed a significantly higher ARA content in serum PLs, but not in serum TGs, in the group supplemented with ARA than in the control group, after 2 and 4 weeks supplementation. In serum PLs, LA was significantly lower than baseline after 2 and 4 weeks ARA supplementation, while it was unchanged in the control group and in serum TGs. Between group comparison showed no significant difference in LA in serum PLs or TGs. There was no significant change in plasma PL or TG EPA and DHA. This study show that in a healthy male adult population with high fish intake (~860 mg EPA+DHA intake per day) and a basal intake of ~177 mg ARA per day, supplementation with 838 mg/day is able to increase ARA levels in serum PLs without compromising EPA and DHA levels.

Ishikura et al. [24] supplemented the habitual diet of Japanese elderly men with 3 capsules per day providing either 240 mg ARA in the form of ARA-enriched TG (SUNTGA40S; 600 mg oil) or control (600 mg olive oil per day) for 1 month. ARA supplementation significantly increased ARA content in serum PLs (from 140 μg/mL serum to 175 μg/mL serum; this was equivalent to an increase from 8.7% to 10.7% of FAs), and significantly decreased EPA but did not affect DHA. In the control group, there was no change in ARA, EPA or DHA levels. No between group comparison was made.

Hirota et al. [23] performed a double-blind, random order cross-over, placebo-controlled intervention study with 23 young Japanese women aged 18-23 years. The subjects received one 200 mg capsule daily, either containing a low dose of ARA (~80 mg per day in 200 mg ARA enriched-TG) or olive oil as control. The study duration was 12 weeks in total, including 4 successive periods of 3 weeks: 3 weeks washout, 3 weeks with treatment 1, 3 weeks washout and 3 weeks with treatment 2. The participants were asked to limit their fish consumption to a maximum of four times per week. They recorded their food intake throughout the study, allowing their FA intake at the beginning and at the end of the 2 treatment periods to be estimated. Fasted blood was taken at the beginning (baseline value) and at the end of the two 3 week treatment periods and FAs were analyzed in RBCs and plasma PLs, TGs and CEs. ARA supplementation significantly increased ARA and total n-6 LC-PUFA levels in the 4 blood pools compared to olive oil, without decreasing n-3 LC-PUFAs, except for a significant decrease in n-3 LC-PUFAs in plasma CEs. This study shows that in a female population with relatively high fish intake (460-560 mg EPA + DHA intake per day) and a basal intake of ~150 mg ARA per day, supplementation with a small dose of ARA (~80 mg/day) is able to significantly increase ARA levels in RBCs and plasma fractions without compromising n-3 LC-PUFA levels.

Kakutani et al. [25] performed a double-blind, parallel, placebo-controlled intervention study in 118 healthy Japanese elderly who were not supplement consumers. They received ten 170 mg capsules daily, either containing a low dose or a high dose of ARA (total of ~240 mg or ~720 mg per day) as an ARA enriched-TG (SUNTGA40S) or olive oil as control for 4 weeks, followed by a 4-week washout period. The participants recorded their food intake throughout the study, allowing calculating their FA intake at the baseline and after 4 weeks of supplementation. FAs in plasma PLs were measured in fasted blood at four time points: baseline, after 2 and 4 weeks of supplementation and 4 weeks after the end of

supplementation. Supplementation with 240 and 720 mg/day of ARA increased ARA content of plasma PLs by 2.5% and 5.6% of total FAs, respectively, which was significantly different from baseline, and then ARA decreased to a level close to that of baseline 4 weeks after the end of supplementation. Plasma ARA did not increase in the control group. Between group comparison was done but not clearly reported. The ARA increase in plasma PLs was dose dependent, at least up to 720 mg/day. In the high ARA group, plasma PL LA was significantly lower than at baseline after 2 and 4 weeks supplementation, while it was unchanged in the low ARA and the control groups. There was no significant change in plasma PL EPA and DHA. This study shows that in an elderly population with high fish intake (853-1176 mg EPA+DHA intake per day) and a basal intake of ~170 mg ARA per day, supplementation with a 240 or 720 mg/day is able to dose-dependently increase ARA levels in plasma PLs without compromising EPA and DHA levels.

Recently Markworth et al. [28] reported from a randomized, controlled trial of 1500 mg ARA/day for 4 weeks in young men participating in a resistance training program. FAs were measured in plasma and in skeletal muscle at the start and end of the supplementation period. Plasma ARA increased from 8.4 to 16.2% of total FAs and was higher in the ARA group than in the control group at the end of the intervention. Plasma LA decreased from 25 to 14% of total FAs in the ARA group. Plasma EPA decreased slightly in the ARA group but was not different between groups at week 4. Plasma DHA was not significantly altered. Skeletal muscle ARA increased from 12 to 14.6% of total FAs but this was not a significant effect.

Smit et al. [33] performed a randomized, open intervention study in 20 breastfeeding Israeli women. The women's mean age was 23 years and the lactation duration ranged between 3 and 10 months. The ARA group received 284 mg ARA per day (0.8 ml ARA-rich oil) while the control group did not receive any supplement. The study duration was 1 week, during which 3 milk samples were taken (before, after 1 day and after 7 days supplementation). Milk FAs were analyzed and the FA composition of the 2 groups was compared at the 3 time-points. It was found that women in the ARA group had the same content of ARA in their milk as women who did not receive any supplement. Other long chain n-6 PUFAs and DHA did not differ either, while EPA was significantly lower in the ARA group at day 7. This small study suggests that in a population of lactating women with a habitual estimated ARA intake of ~200 mg per day, a supplement of ~300 mg ARA per day does not significantly affect milk ARA (or DHA), but does lower milk EPA. This effect can be observed after one week of ARA supplementation.

In Egyptian schoolchildren infected with *Schistosoma mansoni*, ARA (10 mg/kg body weight per day for 5 days in each of 3 weeks) significantly increased plasma ARA from 7.9 to 12.1% of total FAs, with no significant effect on LA [20].

In summary, increased ARA intake significantly increases the ARA content in different blood fractions, including RBCs, platelets and mononuclear cells, with doses as low as 80 mg per day being effective. The low dose of 40 mg ARA per day did not affect ARA level in human milk. It is likely that enrichment of ARA in different compartments and pools is dose-dependent. Figure 5 shows data for serum and plasma PLs from seven studies and indicates a near linear dose-dependent increase in ARA content up to an intake of about 1000 mg/day. Lack of studies using higher intakes of ARA and the short duration of most studies performed to date limits a full understanding of this dose-response relationship. A study specifically designed to assess the dose-response relationship is required to be certain about the exact nature of this relationship. EPA was decreased in several studies (human milk with 40 mg/day ARA, plasma CEs with 80 mg/day ARA, serum PLs with 240 mg/day ARA, plasma with 1500 mg/day ARA) and one study reported a decrease in DHA content of RBCs when a high dose (1.7 g/day) of ARA was consumed for 50 days.

Effect of ARA on PUFA metabolism

A small sub-study [22] within the larger study by Nelson et al. [13, 14] investigated the effect of ARA on Δ5-desaturation and incorporation of deuterium-labeled dihomo-gamma-linolenic acid (DGLA; 20:3n-6) into plasma lipids. Adult male subjects (n = 4) were given diets containing either 1.7 g/day ARA or 0.21 g/day ARA for 50 days. After 50 days, subjects were dosed with a mixture containing deuterium labelled ethyl esters of DGLA[d4] and oleic acid[d2] and a series of blood samples were sequentially drawn over a 72 h period. The estimated conversion of DGLA[d4] to ARA[d4] was 17.7 ± 0.8% when subjects had been on the high ARA intake and 2.1 ± 1.4% when subjects had been on the low ARA intake. The concentrations of ARA[d4] in total plasma lipids from subjects after the high and low ARA periods were 2.1 ± 0.6 and 0.3 ± 0.2 μmol/ml plasma/mmol of DGLA[d4] fed/kg of body weight. These data indicate that conversion of DGLA[d4] to ARA[d4] was stimulated 7 to 8-fold by the high ARA intake, although a decrease in turnover of ARA in the high ARA group cannot be excluded.

Effect of ARA on blood lipid concentrations

Roberts et al. [30] conducted a study in resistance-trained male subjects and found that serum cholesterol concentrations were not changed after ARA-supplementation (1 g/day for 50 days). Likewise, there were no changes in serum concentrations of total, low-density and high-density lipoprotein cholesterol, TGs, apolipoprotein AI, apolipoprotein B, or lipoprotein [a] after ARA supplementation in different study designs (see Table 2) in adults [14, 26, 28] and school children [20, 32].

Thus, from the available evidence it appears that increasing ARA intake even up to 1.7 g/day does not affect blood lipid concentrations. However, there are few such studies and the effect of ARA on blood lipids in dyslipidemic subjects has not been investigated.

Effect of ARA on blood pressure

In the study of Kusumoto et al. [26] blood pressure was not affected by increasing ARA intake to 838 mg/day. Participants in this study were normotensive and there are no studies of increasing ARA intake on blood pressure in hypertensive subjects.

Effect of ARA on platelet aggregation, bleeding and haematological markers

In a randomized double-blind trial in Italian patients with liver cirrhosis, Pantaleo et al. [29] compared the effects of 2 g/day ARA from ARASCO for 8 weeks with those of oleic acid on collagen-induced aggregation of platelet-rich plasma. Compared with pre-study levels, platelet aggregation was significantly increased in the ARA group, but not in the oleic acid group.

Kusumoto et al. [26] carried out a double-blind, placebo-controlled study in healthy Japanese men consuming a high-fish diet. One group of men received capsules providing 838 mg ARA incorporated into TG (SUNTGA40S) daily for 4 weeks, while another group received capsules with olive oil as control. Collagen-, ARA- and ADP-induced aggregation of platelet-rich plasma were not affected by ARA supplementation. Also, haematological parameters (haemoglobin concentration, packed cell volume, RBC numbers, total leucocytes, thrombocytes, and mean of corpuscular volume) and coagulation parameters (prothrombin time and antithrombin III activity) remained unchanged.

In a single-blind cross-over study, healthy male volunteers received 1.7 g/day or 0.2 g/day of ARA for 50 days [13]. Aggregation of platelet-rich plasma was induced using ADP, collagen, and ARA. No effects of increased ARA intake were observed. Also, platelet count, bleeding

time, partial thromboplastin time, and antithrombin III concentrations remained unchanged. Prothrombin time, however, was significantly lowered by about 10% after ARA intake.

In a randomized double-blind study, resistance-trained male subjects received either ARA (1 g/day) or corn oil as placebo for 50 days [30]. There was no effect on RBC numbers.

Most haematological parameters were not altered by 1500 mg/day ARA for 4 weeks in healthy young men [28].

Haematological parameters (haemoglobin concentration, packed cell volume, RBC numbers, numbers of segmented neutrophils, eosinophils, basophils and platelets) were not changed in a study with Egyptian school children who received ARA (10 mg/kg body weight per day) for 15 days (5 days over each of 3 consecutive weeks) with or without praziquantel, a medication used to treat certain types of parasitic worm infection [20]. Several coagulation parameters (prothrombin concentration, international normalized ratio, and partial thromboplastin time) were also not changed, although both prothrombin time and clotting time were significantly shorter, although only by < 2% [20]. Comparable results were found in a smaller study in older school children using a similar design [32].

In summary, most studies report no effect of increased ARA intake on platelet aggregation or coagulation parameters and no studies report effects on bleeding time. However, the study that used the highest intake of ARA [29] reported enhanced platelet aggregation. The early study of Seyberth et al. [12] using 6 g ARA/day also saw this effect. The effect of doses of ARA of 2 g/day or more on platelet aggregation requires further investigation.

Effect of ARA on biomarkers of immunity and inflammation

Kelley et al. [15, 16] reported data from a controlled, cross-over trial in 10 healthy adult men in the US; this is the same study as Nelson et al. [13, 14]. The participants consumed a standard diet providing 0.21 g ARA/day or an intervention diet providing 1.7 g ARA/day for 50 days. ARA did not influence ex vivo lymphocyte proliferation in response to several agents or ex vivo natural killer cell activity [15]. Participants received the measles/mumps/rubella and influenza vaccines: the ex vivo proliferation of lymphocytes in response to influenza vaccine was about four-fold higher in the group receiving 1.7 g ARA/day than in the low ARA group [15]. However, serum antibody titres against the influenza vaccine were not affected by high ARA [16]. Although the total number of white blood cells (leukocytes) was not affected, there

were significantly more blood granulocytes (mainly neutrophils) when participants received 1.7 g ARA/day [15]. However, blood lymphocyte and monocyte numbers were not affected by ARA [16]. Ex vivo production of several inflammatory cytokines in response to lipopolysaccharide was not different between low and high ARA treatment [16]. However, lipopolysaccharide-stimulated production of PGE_2 and leukotriene B_4 was significantly higher after high ARA than after the standard diet [16]. This probably reflects the higher ARA content of the cells involved, although this was not reported.

Thies et al. [34-36] reported results from a placebo-controlled, randomised, parallel study in healthy older British subjects: 8 participants received a blend of palm and sunflower seed oils as control while 8 received ~700 mg ARA (from ARASCO) daily for 12 weeks. Participant's habitual intake of ARA was about 150 mg/day. Blood samples were collected at baseline and at 4, 8 and 12 weeks supplementation and then after 4 weeks washout. There was no effect of increased intake of ARA on the numbers or percentages of different types of immune cells in the blood, on ex vivo blood lymphocyte proliferation in response to mitogenic stimulation, on ex vivo production of T-cell derived cytokines or lipopolysaccharide-stimulated cytokines, on ex vivo natural killer cell activity, on phagocytic activity and respiratory burst of neutrophils and monocytes, or on the plasma concentrations of three different adhesion molecules [34-36]. This study clearly demonstrates that there is no strong impact of ~700 mg/day ARA on blood immune cell numbers, on ex vivo markers of the immune response, or on markers of inflammation in healthy older subjects. The study did not measure concentrations or production of ARA-derived lipid mediators like PGs.

Roberts et al. [30] saw no effect of 1 g ARA/day for 50 days on total blood leukocyte numbers or types in resistance trained men.

Schubert et al. [31] performed a study comparing a mix of FAs considered to be anti-inflammatory with ARA (40 mg/day) for 2 weeks in 30 healthy participants; an additional blood sample was collected 2 weeks after stopping the intervention. Whole blood was stimulated ex vivo with lipopolysaccharide and appearance of PGE_1 (produced from DGLA), PGE_2, leukotriene B_4, tumour necrosis factor, interleukin 8 and interleukin 10 were measured. There were no significant differences in any of these at the end of supplementation compared to baseline in either group and there were no significant differences between the two groups at the end of the supplementation period. However, some differences were seen between groups 2 weeks after stopping the supplementation; these are difficult to interpret.

Kakutani et al. [25] performed a double-blinded, parallel, placebo-controlled intervention study with 118 healthy Japanese elderly subjects. They received olive oil as control or capsules providing ~240 mg or ~720 mg ARA/day for 4 weeks, followed by a 4-week washout period. There was no impact of ARA on circulating concentrations of C-reactive protein, immunoglobulin E, two pro-inflammatory cytokines (tumour necrosis factor and interleukin-6), PGE_2 or lipoxin A_4. The authors concluded that there was no impact of 240 or 720 mg ARA daily on inflammation.

In Egyptian schoolchildren infected with *S. mansoni*, ARA (10 mg/kg body weight per day for 5 days in each of 3 weeks) significantly decreased plasma interleukin-10 and interferon-γ concentrations compared with study entry [20]. However, these findings are difficult to interpret because ARA was able to effectively treat the parasitic infection and the altered plasma cytokines may simply reflect reduced pathogen burden.

In summary, the available evidence suggests little or no impact of increasing ARA intake by as much as 1.5 g/day on immune function or on markers of inflammation, apart from a small increase in ARA-derived eicosanoid production when cells are stimulated. However, the health impact of the latter response is not known.

Effect of ARA on cognitive function

Despite ARA having an important structural and functional role in the brain [7], there are very few RCTs of cognitive function in humans where ARA is the sole intervention. Most intervention studies involving ARA have been in combination with DHA and have been undertaken during infancy.

Ishikura et al. [24] investigated the effects of ARA on age-related event-related potentials in 25 healthy elderly Japanese men. The study was performed using a double-blind crossover design and the subjects were administered 240 mg/day of ARA from an ARA-enriched TG (SUNTGA40S) in capsules or the same amount of olive oil in capsules as placebo for 1 month. Event-related potentials, which included P300 latency and amplitude, were measured before capsule administration and after 1 month of administration. In subjects administered ARA, P300 latency was significantly shorter, and P300 amplitude was significantly higher than in those administered olive oil capsules. It was concluded that supplementation of ARA may reduce age-related decline in cognitive function and learning ability. However, this is based upon a single small study and more research is needed in this important area.

484

485 ***Effect of ARA on body composition, muscle function and physical performance***

486 Three double blind RCTs have reported outcomes of the effect of ARA on body composition,
487 muscle function and physical performance [21, 27, 30]. In the first study, 31 males from the US
488 were randomly assigned to receive capsules providing either 1000 mg ARA or corn oil per
489 day for 50 days. No significant effects were found on body weight, fat free mass, fat mass,
490 anabolic hormones, or intramuscular markers of muscle hypertrophy [30]. However compared
491 to baseline, ARA supplementation increased anaerobic peak power by 8.5% at day 50. On
492 day 25, the ARA supplemented group had attenuated serum interleukin-6 levels whereas
493 levels of serum PGE_2, a potential ergogenic factor, tended towards an increase. The authors
494 suggested that ARA supplementation would decrease inflammation (lower interleukin-6),
495 thus making intense training more tolerable. No support was found for ARA to stimulate
496 muscle hypertrophy, which would lead to a greater strength gain and/or muscle mass due to
497 training.

498

499 In the second study, 30 males from the US were randomized to receive either 600 mg ARA
500 (from 1.5 g ARASYN oil) or corn oil daily during an eight week training program. The ARA
501 group showed a significant increase in lean body mass (2.9%), upper body strength (8.7%)
502 and peak power (12.7%) [21]. ARA supplementation was suggested to increase post-exercise
503 anabolic signaling rather than protein synthesis in skeletal muscle.

504

505 Markworth et al. [28] conducted a 4-week RCT of 1500 mg/day ARA in 19 young males
506 involved in a resistance training programme. There was a significant reduction in fat mass (-
507 0.33 kg or -1.7%) in the ARA group compared to the control group (+0.49 kg or +3.8%).
508 There was no effect of ARA on lean mass and effects of ARA on leg muscle volume were
509 small. Other measurements in these individuals were reported by Mitchell et al. [27]: prior
510 ARA supplementation did not alter the acute anabolic response (i.e. muscle protein synthesis
511 and anabolic signaling in muscle) to resistance exercise in these trained men, and the authors
512 concluded that there is no link between ARA and short-term anabolism. However, some
513 muscle changes were seen 48 hours after completing the resistance exercise in men in the
514 ARA group.

515

In summary, two of these studies suggest that ARA can improve peak power and may have an effect on lean body mass while a third study suggests that ARA has an effect on late responses to resistance exercise. These effects and the underlying mechanisms require further exploration.

Effect of ARA on urinary metabolites

Ferretti et al. [17] reported increased urinary levels of ARA metabolites (11-dehydro-thromboxane B_2 and 2,3-dinor-6-oxo-$PGF_{1\alpha}$) following 1.7 g ARA/day for 50 days. Kidney diuretic function, both normal and diuretic-stimulated, was not compromised in patients with liver cirrhosis (aged > 60 years) who consumed 2 g/day ARA for 8 weeks: urinary sodium and ARA metabolites (i.e. PGE_2, 6-keto-$PGF_{1\alpha}$, 8-epi-$PGF_{2\alpha}$ and 11-dehydro-thromboxane B_2) were similar to those observed in the placebo group [29]. More recently, with smaller dosages (240 or 720 mg ARA/day), for a shorter duration (4 weeks), and on a background of high DHA and EPA intake levels, ARA metabolite (11-dehydro-thromboxane B_2, 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid),) excretion in healthy Japanese elderly (>55 years of age) were also found not to be affected compared to the placebo (olive oil) group [25].

In summary, most studies report no effect of increased ARA intake on urinary excretion of ARA metabolites and one study reports no effect of ARA on renal function in cirrhotic patients.

Summary and conclusions

The literature search identified 22 articles from 14 RCTs of increasing ARA intake in humans. These studies were published between 1997 and 2018. Most were conducted in adults. Studies in adults used between 40 and 2000 mg ARA/day, were of 1 to 12 weeks duration and usually used ARA as a supplement. Most studies used ARA intakes of 240 to 1000 mg/day. Only one study investigated more than one dose of ARA [25]. Many studies were conducted in healthy young or older subjects and several were restricted to men. One study was conducted in breast feeding women and one in patients with liver cirrhosis. Few studies controlled the diet of the subjects under study and few studies assessed background diet. A number of studies reported the effect of ARA on FA composition of blood pools like lipids, RBCs and mononuclear cells and one study reported on breast milk FAs and another on skeletal muscle FAs. Given the role of ARA-derived eicosanoids in regulating

549 inflammation, immune function, platelet aggregation and blood clotting, it is not surprising
550 that several studies investigated the effect of ARA on these outcomes. In contrast, there have
551 been only few studies investigating effects on blood lipids, blood pressure, and cognition. In
552 most of these areas there are too few studies to draw firm conclusions on the impact of ARA.
553 Furthermore, the risk of bias was unclear for many of the studies, limiting the robustness of
554 their findings.

555 It is clear from the existing studies that ARA supplements significantly increase the content
556 of ARA in different blood fractions with doses as low as 80 mg per day being effective. The
557 low dose of 40 mg ARA per day did not affect ARA level in human milk. It is likely that
558 enrichment of ARA in different compartments and pools is dose-dependent, but may become
559 saturated at higher intakes. EPA was decreased in several studies but DHA was usually not
560 affected by ARA supplementation, even at the highest doses tested. Often incorporation of
561 ARA was at the expense of LA. From the available evidence, it appears that increasing ARA
562 intake does not affect blood lipid concentrations or blood pressure. However, there are few
563 such studies and the effect of ARA on blood lipids in dyslipidemic subjects or on blood
564 pressure in hypertensive subjects has not been investigated. Furthermore, most studies have
565 reported no effect of increased ARA intake on platelet aggregation or coagulation parameters
566 and no studies have seen effects on bleeding time. However, one study that used the highest
567 intake of ARA [(29)] reported enhanced platelet aggregation and this requires further
568 investigation. The available evidence from rather detailed studies suggests little or no impact
569 of increasing ARA intake by as much as 1.5 g/d on immune function or on markers of
570 inflammation, apart from a small increase in ARA-derived eicosanoid production when
571 immune cells are stimulated. However, the effect of the latter response is not known. Several
572 studies report no effect of increased ARA intake on urinary excretion of ARA metabolites.
573 One study concluded that supplementation with ARA may reduce age-related decline in
574 cognitive function and learning ability. This could be an important effect and more research
575 should be conducted in this area. Another interesting observation is the improvement in peak
576 power and lean body mass seen in young adults undergoing exercise training. Again these
577 effects need further investigation along with exploration of the likely mechanisms. It is
578 important to note that none of the studies included here was of more than 12 weeks duration,
579 and most were shorter than this; this may be insufficient time to affect several of the
580 outcomes assessed such as cognitive function, body composition and physical performance.
581

Thus, overall there seem to be few marked benefits of increasing ARA intake from the typical intake of 100-200 mg/day to as much as 1000 mg/day or perhaps even more. However, the suggested impacts on cognitive and muscle function may both be important, particularly in the ageing population, and therefore the effect of higher intakes of ARA is deserving of further study. The studies reviewed herein suggest no adverse effects of increased ARA intake up to at least 1000 to 1500 mg/day on blood lipids, platelet aggregation and blood clotting, immune function, inflammation, or urinary excretion of ARA metabolites. However, in many areas there are insufficient studies to make firm conclusions. Based on the RCTs reviewed, there are not enough data to make any recommendations for specific health effects of ARA intake

Authors contributions

All authors conceived the research, selected papers for inclusion, carried out data extraction and assessment of papers, and contributed to drafting of the manuscript. SL and AS conducted the literature search. PCC had responsibility for final preparation of the manuscript. All authors have read and approved the final version of the manuscript.

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Ans Eilander works for Unilever; Mathilde Fleith works for Nestle; Per-Olof Larsson works for BASF; Bert van de Heijning works for Danone; Stewart Forsyth consults for DSM; Philip Calder consults for DSM, Danone/Nutricia, Cargill, BASF, FrieslandCampina and Smartfish; ILSI Europe is funded by its industry members.

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Figure captions

Figure 1. Structure of arachidonic acid. The numbers 1, 5, 8, 11 and 14 beneath the hydrocarbon chain refer to carbon number counting from the α end of the chain. The numbers 1 and 6 above the hydrocarbon chain refer to carbon number counting from the ω end of the chain.

Figure 2. Outline of the pathway of biosynthesis and further metabolism of arachidonic acid.

Figure 3. PRISMA flow-chart of study selection.

Figure 4. Risk of bias analyses. Under 'other bias' power analyses, statistical shortcomings (e.g. only within treatment comparisons) and experimental design issues were considered. Legend: green: low risk of bias; red: high risk of bias, orange: unclear risk of bias.

Figure 5. Relationship between arachidonic acid (ARA) intake (mg/day) and increment in ARA in serum or plasma phospholipids (as % of total fatty acids). Data are taken from Hirota et al. [23], Ishikura et al. [24], Kakutani et al. [25] [two doses of ARA used], Kusumoto et al. [26], Nelson et al. [14], Pantaleo et al. [29] and Thies et al. [34]. The line of best fit for these data points is shown.

Table 1. Search terms used for the Cochrane Central Database search.

1	exp Arachidonic Acid/ (18143)
2	arachidonic acid*.mp. (46543)
3	arachidonate*.mp. (11283)
4	eicosatetraenoic acid*.mp. (3825)
5	eicosatetranoic acid*.mp. (22)
6	20:4 n-6.mp. (932)
7	20:4n-6.mp. (986)
8	20:4n6.mp. (101)
9	or/1-8 (53238)
10	randomi?ed controlled trial.pt. (469845)
11	controlled clinical trial.pt. (94452)
12	randomi?ed.ab. (411841)
13	placebo.ab. (191566)
14	clinical trials as topic.sh. (187518)
15	randomly.ab. (285414)
16	trial.ti. (184903)
17	or/10-16 (1153410)
18	exp animals/ not humans.sh. (4442323)
19	17 not 18 (1063843)
20	9 and 19 (1502)