Do omega-3 polyunsaturated fatty acids have a beneficial effect on recovery from exercise in trained males: a systematic review

Word Count: 9975

Declaration of Originality

I confirm that this assignment is my own work and that I have:

- Read and understood the guidance on plagiarism.
- Clearly referenced, in both the text and the bibliography or references, all sources used in the work.
- Fully referenced (including page numbers) and used inverted commas for all text quoted from books, journals, web etc.
- Provided the sources for all tables, figures, data etc. that are not my own work.
- Not made use of the work of any other student(s) past or present without acknowledgement.
- This includes any of my own work, that has been previously, or concurrently, submitted for assessment, either at this or any other educational institution, including school.

Declaration:

I am aware of and understand the University's policy on plagiarism and I certify that this assignment is my own work, except where indicated by referencing.

Date: 08/ 06/ 17

Acknowledgements

TABLE OF CONTENTS

ABBREVIATIONS

AA	Arachidonic acid
ALA	α-Linolenic acid
ALT	Alanine Transaminase
AST	Aspartate Transaminase
$-CH_3$	Methyl group
CK	Creatine Kinase
CONSORT	Consolidated Standards of Reporting Trials
COOH	Carboxyl group
COX	Cyclooxygenase enzymes
CRP	C-reactive protein
DHA	Docosahexanoic Acid
DOMS	Delayed Onset Muscle Soreness
DPA	Docosapentaenoic acid
EIMD	Exericse Induced Muscle Damage
EMBASE	Excerpta Medica dataBASE
EPA	Eicosapentanoic Acid
FRAP	Ferric Reducing Antioxidant Power
γGT (GGT)	Gamma-Glutamyl Transpeptidase
IL-1RA	Interleukin-1 Receptor Antagonist

IL-2	Interleukin-2
IL-6	Interluekin-6
IL-8	Interleukin-8
LDH	Lactate Dehydrogenase
LOX	Lipoxygenase enzymes
MDA	Malondialdehyde
MEDLINE	Medical Literature Analysis and Retrieval System Online
ORAC	Oxygen Radical Absorbance Capacity
PGE2	Prostaglandin E2
PUFAs	Polyunsaturated Fatty Acids
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RDA	Recommended Daily Allowance
TGF-β	Transforming Growth Factor beta
TNF- α	Tumour Necrosis Factor alpha
TTE	Time To Exhaustion

GLOSSARY

mg	Milligrams

ABSTRACT

Aim: To critically appraise the evidence in relation to the benefits of omega-3 polyunsaturated fatty acids on recovery from exercise amongst trained males. Study design, participant background, type of exercise, and supplementation protocol were evaluated.

Methods: Ovid Medline, Web of Science and Pub Med were searched. Inclusion and exclusion criteria were applied resulting in 14 papers; all of which were randomised control trials.

Results: The number of participants ranged from 13 to 48, from a variety of sporting backgrounds. Different exercise protocols were performed in order to induce muscular damage and / or inflammation. Many different outcomes were measured and overall ten studies found a beneficial effect while four studies found no effect.

Discussion: Evidence suggests that omega-3 PUFA supplementation has beneficial effects on multiple post exercise outcomes. Disagreement between studies may be explained by the heterogeneity of design or limitations in their methodology. The potential for practical application of benefits is largely dependent on the type of exercise and indeed whether biochemical outcomes would translate into improved performance.

Limitations: Time and resources were limited meaning the search methodology could not be verified. This study was also underpowered to conduct a meta-analysis of results, which may have proven statistical significance of the combined studies.

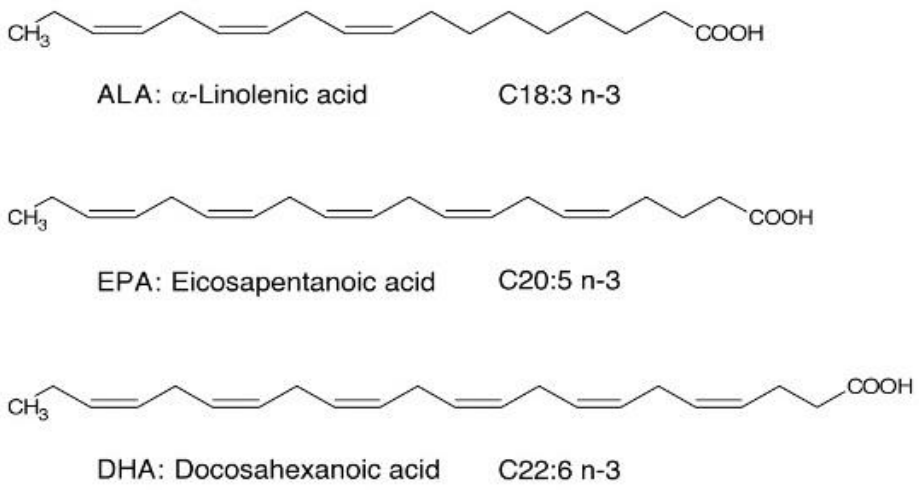
Recommendations: Future studies should focus on practical applications of improvements in performance and attempting to determine the optimal supplementation protocol.

1 INTRODUCTION

1.1 What are Polyunsaturated Fatty Acids (PUFAs)

PUFAs are organic acids consisting of a hydrocarbon backbone with carboxyl group (COOH) at one end (alpha end) and a methyl group (-CH_3) at the other (omega end). They are termed polyunsaturated because there are at least two double (unsaturated) bonds between adjoining carbons atoms in the hydrocarbon chain. Omega-3 PUFAs differ from omega-6 PUFAs by having the first double bond between the third and fourth carbons from the omega end, rather than the sixth and seventh carbons. These double bonds allow for the easy oxidation and /or addition of further hydrogen atoms, making omega-3 an unstable form of fat. Such oxidised versions of omega three have been implicated in both ageing (Simopoulos 2002) and inflammatory processes (Li et al. 2014) and thus the pathogenesis of many diseases (Simopoulos 2008). The structure of the three key omega-3 PUFAs, α-Linolenic acid (ALA), Eicosapentanoic acid (EPA) and Docosahexanoic acid (DHA) are shown below in Figure 1.

Figure 1: Structure Omega-3 Polyunsaturated Fatty Acids

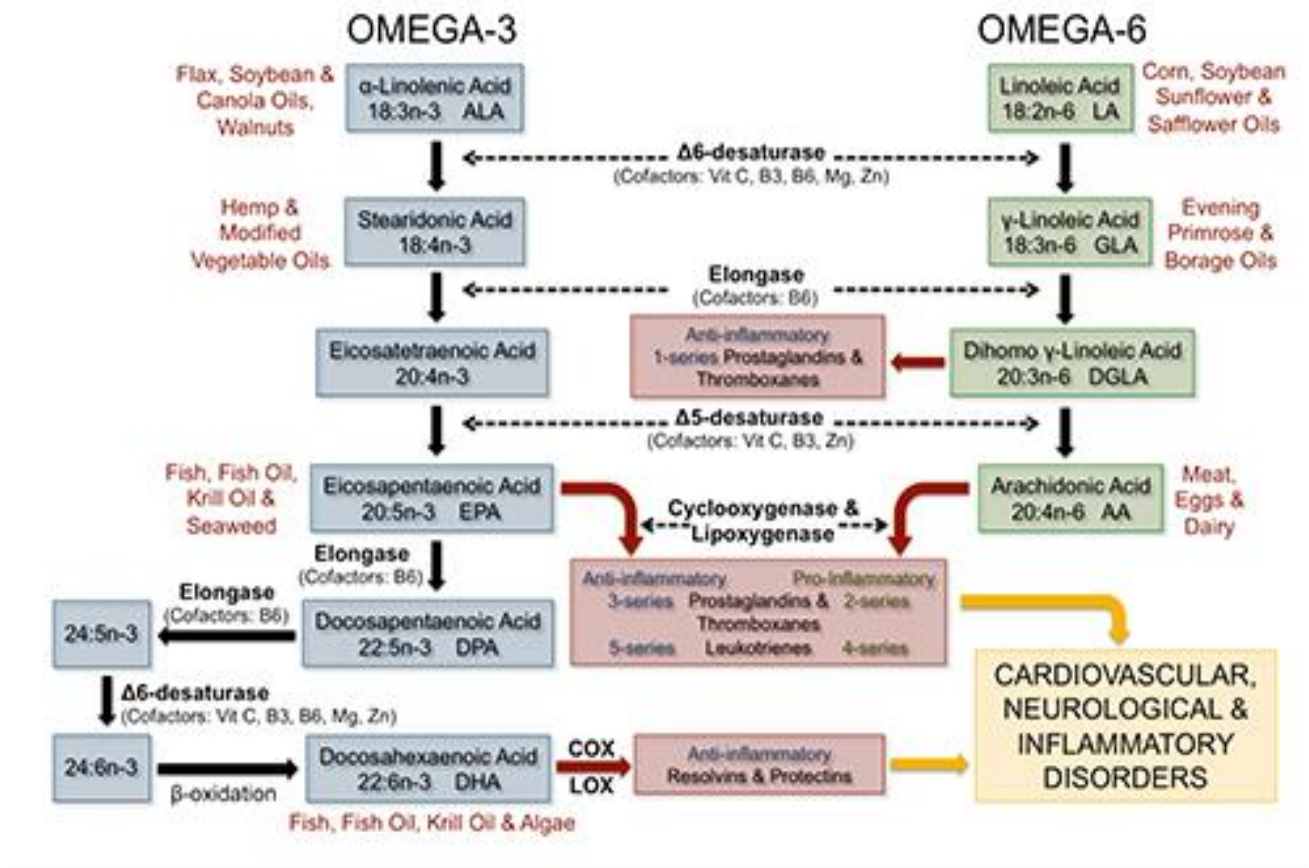


Omega-3 PUFAs are referred to as essential fatty acids, because they cannot be synthesised within the human body and therefore, must be obtained from diet. Notable sources of omega-3 PUFAs include fish, vegetable oil (rapeseed, linseed) and nuts and seeds, and their derivative nut oils, for example walnuts (Kris-Etherton et al. 2002). Most omega-3 PUFAs that are consumed in this way are shorter chain molecules, such as ALA. However, the most important are EPA and DHA; but only a small amount of total ALA (around 5%) is converted in EPA and DHA. The National Institute for Health and Clinical Excellence (NICE) suggest consuming two portions of fish per week (NICE 2008), equating to approximately 500mg of EPA + DHA per day. As a result of the inefficiency of the in vivo synthesis, in order for humans to meet this recommended daily allowance (RDA), the majority of these particular omega-3 PUFAs must come from the diet. Foods highest in these essential fatty acids include oily fish, particularly salmon, herring, and mackerel. However, obtaining EPA and DHA from dietary supplements alone is also sufficient to meet the body's requirements.

1.2 Role of Omega-3 PUFAs

The role of individual fatty acids is dependent on several factors including the size of the molecule, along with the number and location of double bonds. Therefore, due to the placement of the first double bonds, omega-3 and omega-6 PUFAs exert different effects in the body. In their original form fatty acids provide the basis for the lipid bilayer of cell membranes. However, it is the interconversion of short chain omega-3 to longer chain fatty acids, as summarised in Figure 2, that is responsible for their wide range of effects.

Figure 2: Derivatives of omega-3 and omega-6 PUFAs



Modification of dietary PUFA's involves the addition of carbon atoms to the hydrogen backbone, resulting in longer chains of PUFAs. The same enzymes work on both omega-3 and omega-6 PUFAs, although the various desaturase and elongase enzymes act preferentially on omega-3 PUFA chains. Despite this, increased intake of omega-6 PUFAs can reduce the synthesis of EPA and DHA from ALA.

Once longer chains are synthesised, these PUFAs can be further modified by enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX) to create a series of organic signalling molecules known as eicosanoids. The most prominent sub groups are prostaglandins, thromboxanes and leukotrienes but within these groups, there are different series of metabolites. Prostaglandins and thromboxanes derived from omega-6 PUFAs (series 2 and 4) are aggressively pro-inflammatory; whilst those from omega-3

PUFAs (series 3 and 5) are less inflammatory, inactive, or even anti-inflammatory. Niu et al. (2006) demonstrates this in a cross sectional study showing a diet high in marine oils lowered CRP, a commonly used blood marker for inflammation.

The anti-inflammatory effects of omega-3, and the importance of the balance between omega-3 and omega-6 have been investigated for a number of cardiovascular, inflammatory, and autoimmune diseases (Simopoulos 2008).

1.3 Impact on health

Humans evolved on a diet containing roughly equal ratios of omega-3 PUFAs and omega-6 PUFAs. However, in current Western diets the ratio is somewhere between 1:12 to 1:25. The cardiovascular health benefits of omega-3 PUFAs were first suggested nearly 40 years ago (Dyerberg et al. 1978), after epidemiological studies observed a low incidence of myocardial infarctions in Greenland Eskimos with a high amount of fish in their diet (Bang et al. 1971).

Research has since continued, linking increased dietary intake of omega-3 PUFAs to a number of beneficial effects on health and wellbeing. Meta analyses have also demonstrated benefits in many inflammatory conditions such as rheumatoid arthritis (Fortin et al. 1995) and ulcerative colitis (Aslan & Triadafilopoulos 1992). However, separate Cochrane reviews for asthma (Thien et al. 2002) and cystic fibrosis (Oliver & Watson 2016) did not prove a significant effect. Several studies have found that DHA plays a role in brain development (Simmer et al. 2008; 2011) and function (Helland et al. 2003). In addition there is strong evidence that omega-3 PUFAs may have a potential role in both the aetiology and treatment of mental health conditions (Freeman 2009).

The trend showing a reduction in cardiovascular events with omega-3 PUFA supplementation has also continued. A large randomised controlled trial involving over 11,000 recent myocardial infarction survivors showed a major reduction in cardiovascular events with EPA and DHA supplementation (GISSI 1999). The methodology used within this study set the template for further research to be carried out. More recently an even larger study - The Japan EPA Lipid Intervention Study (JELIS) - showed a significant reduction in major coronary events in hypercholesterolaemic patients in Japan (Yokoyama et al. 2007).

1.4 Link to exercise

The effects of omega-3 have proven cardiovascular benefits (Bucher et al. 2002), due to the reduction in inflammatory eicosanoids derived from arachidonic acid (AA) production (a potent omega-6 PUFA) (Fritsche 2006), and an increase in the anti-inflammatory omega-3 PUFA derivatives. These same mechanisms could in turn be useful for both exercise, and non-exercise induced damage. Exercise induced muscle damage (EIMD) plays an integral role in adaptation and recovery from exercise (Margaritelis et al. 2015). Inflammation is brought about by a combination of mechanical stress and the production of cytokines and free radicals (Webb & Willems 2010). This is accompanied by a rise in acute phase proteins and markers of oxidative stress. Muscle damage, decreased functional strength, and increased perceived muscle soreness are also seen, but have negative effects on performance. On the other hand, chronic exercise results in the repeated bout effect (Starbuck & Eston 2012), whereby symptoms of muscle damage are reduced if similar exercise is carried out. The body then adapts against consistently raised oxidant levels by increasing levels of antioxidants. The already proved cardiovascular benefits of omega-3 PUFAs also play a role in relation to exercise. Vasodilation allows for

improved performance, as increased blood flow permits faster removal of waste products, such as oxygen and nitrogen reactive species, produced during oxidative stress. Omega-3 PUFAs are known to reduce inflammation and may have antioxidant effects due to their inhibition of lipid oxidation; all of which have the potential to enhance recovery from exercise.

The effect of eccentric exercise, when a muscle is actively lengthened with a load (Lorenz & Reiman 2011), on untrained individuals has been explored, but is of little clinical relevance, as it has no practical applications. This is because dietary supplements, including those containing omega-3 PUFAs are targeted to trained individuals. Conversely, the use of supplementation to enhance recovery in athletes is of great importance. Reduced inflammation and the subsequent increased recovery time after exercise can prevent overtraining and the frequency of injuries. The physiological response to exercise differs between genders, with females oxidising a higher proportion of fats than carbohydrates (Tarnopolsky 2000). In addition, measures of cardiovascular function such as heart rate, stroke volume, and oxygen consumption have been shown to be higher in females despite their lower energy expenditure compared to males (Wheatley et al. 2014). Just as there is a marked difference between genders, there is also a disparity in response to exercise between trained and untrained individuals, both acutely and long term. Therefore, in order to reduce confounding factors this review will focus on trained males or athletes. The impact of nutrition on muscular recovery is a popular and well-researched field. However, to date, there have been no systematic reviews focussing on omega-3 PUFAs. Despite numerous trials being conducted, the size of the effect is still unknown. Furthermore, there is some disagreement between the current literature about whether any benefits are significant.

Therefore, the aim of this review is to determine whether, and in what circumstances, omega-3 PUFAs have a beneficial impact on recovery from exercise in trained males.

2 METHODOLOGY

2.1 Search methods for identification of studies

2.1.1 Electronic searches

The following three databases were used as sources of information; Ovid Medline (1946 – 2017), Web of Science (1900 – 2017) and PubMed (1966 – 2017). Advanced searches were carried out using the terms omega 3 AND (exercise OR delayed onset muscle soreness). The Boolean operator NOT was not utilised for the search, as it could potentially omit relevant papers.

These databases were preferentially chosen due to their individual and combined strengths. PubMed focuses on biomedical and clinical journals and is reported to contain the largest number of citations between the databases at 27 million. In addition, in process citations are included, resulting in access to extremely recent papers. Ovid also enables access to MEDLINE (Medical Literature Analysis and Retrieval System Online), plus searching outside it, giving a selection of databases. It also allows the user to build a search strategy in stages, by combining previous steps in the search. Both PubMed and Ovid are updated daily and furthermore use MeSH (Medical Subject Headings) which enables the user to structure their searches using specific concepts. Articles appear tagged with important information about their content and major topics based on the search terms used. Web of Science is a multi-disciplinary database with 256 disciplines, allowing broad searches outside of MEDLINE. Articles can also be sorted by the number of times cited elsewhere, but unlike the other databases utilised they are not tagged for content, so a more extensive search criteria with all possible variations of words was required.

Other databases were also searched, however there was no gain in retrieving further articles from them. Therefore, papers retrieved from them are not included in this review. This could be due to overlap with MEDLINE, such as with EMBASE (Excerpta Medica database).

2.1.2 Reference lists

The references of the final papers that had fulfilled all inclusion criteria were hand searched.

2.2 Selection of studies

After the initial searches were carried out for each database, the searches were documented on a Microsoft Excel spread sheet. Once the search criteria had been finalised, the searches were saved, and downloaded onto two reference management programmes, Endnote and Mendeley. Internal duplicates were removed from within each database, followed by external duplicates between the remaining citations. The steps undertaken in an attempt to deduplicate references were carried out separately in each programme and then hand checked for accuracy. The non-duplicate citations were imported into Covidence, an online resource "selected by Cochrane [in 2015] to become the standard production platform for Cochrane reviews." This technology was used for title and abstract screening. Unrelated papers based on their titles were excluded, and then the inclusion and exclusion criteria were applied for the abstract screening. The final papers were read in full and matched against both the inclusion and exclusion criteria.

Two papers published in the Human Kinetics Journal were not accessible (Hingley et al. 2017; Gray et al. 2014). The publisher was contacted but did not reply, and the University of Manchester inter-library loans service was not able to provide access.

2.3 Criteria for considering studies for this review

2.3.1 Types of studies

For inclusion, studies had to be defined as randomised controlled trials with the use of a placebo or control group. The articles also had to contain an abstract, as this would be required for screening later in the search. There were no restrictions on language, years within which the study was undertaken, or time taken monitoring subjects during the study.

2.3.2 Types of participants

Recruited participants were limited to healthy human adults, and as such all results involving animals or humans under the age of 18 were excluded. Participants were required to either amateur or professional athletes, or be trained in some form of exercise, which had to be defined and explained within the article. Studies involving untrained individuals were excluded. A minimum limit on the sample size of at least 12 participants was set, with at least 6 being required in any supplementation or control group. Anything less than this would not have been a representative sample number. No numerical limit was put on age, although any studies involving specifically mentioning older, elderly, or geriatric participants were excluded. Reviews of a mixed gender cohort would have resulted in unreliable conclusions, as woman may experience a greater degree of muscle damage compared to men. Therefore, studies involving a purely female cohort were also excluded. To eliminate this uncertainty and allow a better comparison, the results of females will not be considered. In addition to this, studies involving postmenopausal women were excluded; this allowed for the exclusion of all female and elderly population studies. Allocation of participants to the treatment groups had to be randomised.

2.3.3 Types of interventions

Omega-3 supplementation in any form had to be given to at least one group within the study. This had to be specifically, either EPA or DHA, but not necessarily both. No limits were set on the frequency, duration, or source of omega-3; however all the above had to be mentioned to warrant inclusion. It has been suggested that an omega-3 dose of greater than 6 g/ day is not a practical daily dose; therefore this formed the maximum limit (Wang et al. 2006). No lower limit was put on dosage. A description of steps taken to ensure omega-3 levels were not inappropriately elevated either through diet or prior supplementation was compulsory.

2.3.4 Exercise protocol

Any study involving exercise was included and no restrictions were put on the type. However, for control purposes, a description of the type of exercise used in the study was required.

2.3.5 Quality assessment

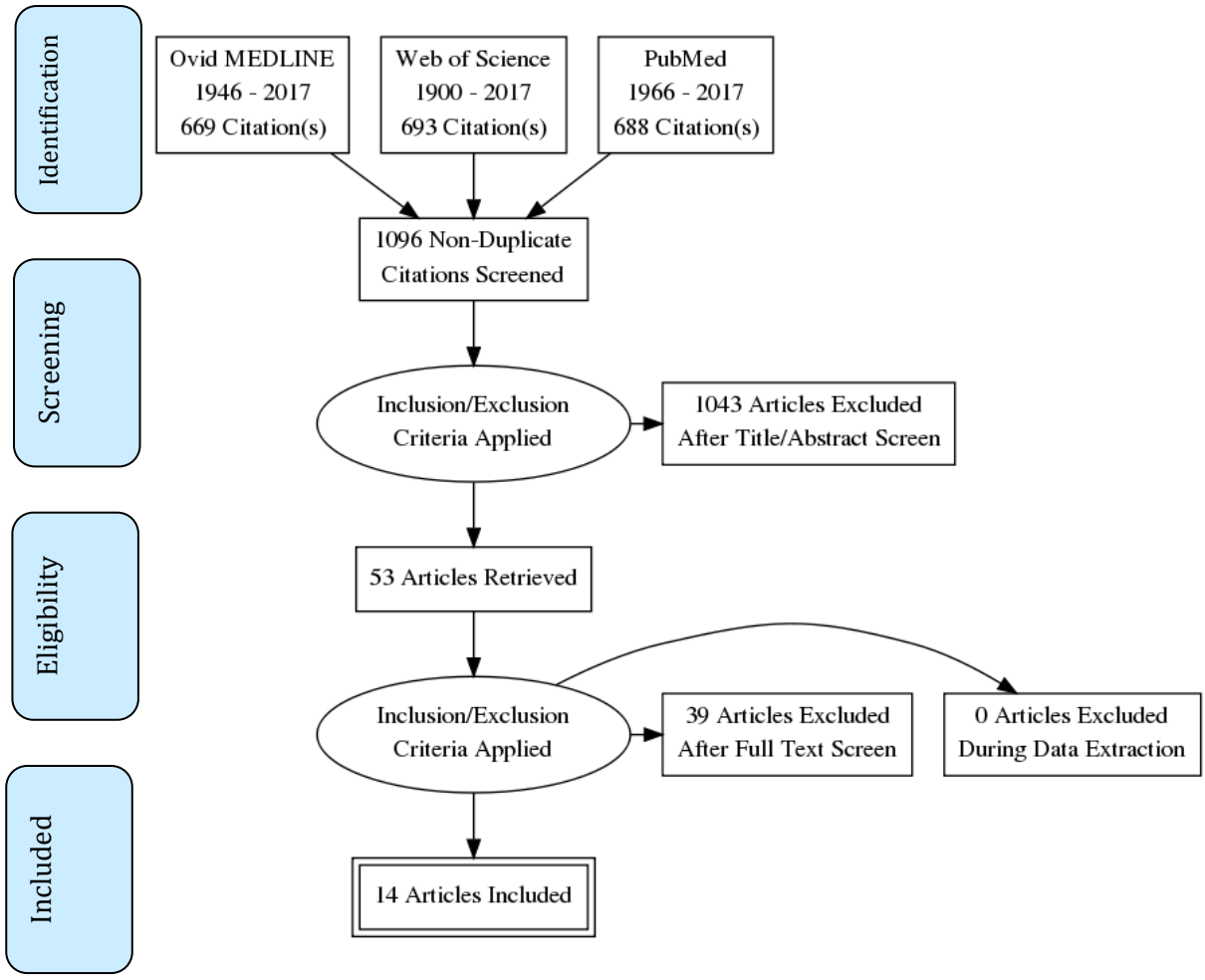
To ensure the quality of any papers to be included, the CONSORT (Consolidated Standard of Reporting Trials) statement was used (Schulz et al. 2010). This comprises a 25-point checklist assessing, but not limited to: background and objectives of the study, eligibility criteria of participants, randomisation and blinding, statistical analysis, and discussion of results.

3 RESULTS

3.1 Results of search

A PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram (Figure 3) depicts the different phases undertaken to identify papers in this systematic review (Moher et al. 2009).

Figure 3 – PRISMA flow diagram



A total of 2050 papers were identified; 669 from Ovid MEDLINE (1946 – 2017), 693 from Web of Science (1900 – 2017), and 688 from PubMed (1996 – 2017). Web of Science and Ovid MEDLINE had 2 and 38 internal duplicates removed respectively, whereas none

were found within PubMed. A further 914 external duplicates between the remaining citations across all three databases were removed, resulting in 1096 non-duplicate citations. Title and abstract screening resulted in the exclusion of 1043 irrelevant papers, leaving 53 articles for full text screening. Review using the CONSORT statement resulted in the exclusion of a further 39 papers, finally leaving 14 papers to be included.

3.2 Excluded studies

The reasons for exclusion after full text screening of 53 papers are shown in Table 1.

Table 1: Reasons for excluding studies

Reason for exclusion	Number of papers excluded
Wrong outcome	16
Wrong patient population	13
Wrong study design	6
Wrong dose	2
Wrong intervention	1
No control group	1
Total number of papers excluded	39

3.3 Description of studies

The effects of omega-3 PUFA supplementation on recovery from exercise for each study is shown in Table 2.

Table 2 – Effects of omega-3 PUFA supplementation on recovery from exercise

Author (year)	Population	Omega-3 PUFA dose (grams / day)	Exercise Type	Duration of Supplementation	Effects of omega-3 PUFAs
Atashak et al. (2013)	Collegiate handball players	0.9	Resistance exercise	1 weeks	CRP ↓ MDA ↓ CK →
Bloomer et al. (2009)	Exercise trained: 3 times per week for 30 minutes	4.432	Weighted walking	6 weeks	CRP ↓ MDA → CK →
Buckley et al. (2009)	Australian rules footballers (Port Adelaide FC)	2.01	Mixed intensity running	5 weeks	TTE → V02 max →
Ghiasvand et al. (2010)	Basketball players	2	Intensive endurance training	6 weeks	IL-2 → TNF-α → MDA ↓ Catalase →

Author (year)	Population	Omega-3 PUFA dose (grams / day)	Exercise Type	Duration of Supplementation	Effects of omega-3 PUFAs
Jakeman et al. (2017)	Physically active: >3 hours per week of HITT	High dose group 1.00 Low dose group 0.250	Plyometric jumps	N/A – Acute dose given	Jump performance ↑ CK → IL-6 → Perceived muscle soreness →
Lewis et al. (2015)	Competed in summer Olympics (rowing, sailing, triathlon, running)	0.375	Resistance exercises	21 days	MVC from dominant quadriceps (measure of peak muscle force) ↑
Martorell et al. (2014)	Professional footballers (Real Mallorca B team)	1.14	Football training	8 weeks	PGE2 ↓ MDA → Catalase → CK → CRP →
Martorell et al. (2015)	Professional footballers (Real Mallorca)	1.14	Football training	8 weeks	MDA → Catalase ↓

Author (year)	Population	Omega-3 PUFA dose (grams / day)	Exercise Type	Duration of Supplementation	Effects of omega-3 PUFAs
McAnulty et al. (2010)	Trained cyclists	2.04	3 hour cycling test	6 weeks	F2-isoprostanes ↑ FRAP → ORAC →
Nieman et al. (2009)	Trained cyclists (local and college clubs)	2.04	3 hour cycling test	6 weeks	CRP → IL-6 → IL-8 → IL-1RA → 10km time trial → V02 max →
Raastad et al. (1997)	Footballers (Norwegian soccer leagues 1. – 3. Divisions)	2.64	Football training	10 weeks	V02 max → AT → TTE →
Santos et al. (2012)	Military personnel (16th Regiment Field Artillery)	0.9	Military boot camp	4 weeks	CRP ↓ CK →

Author (year)	Population	Omega-3 PUFA dose (grams / day)	Exercise Type	Duration of Supplementation	Effects of omega-3 PUFAs
Toft et al. (2000)	Endurance trained athletes	.024	Marathon running	6 weeks	TNF-α ➔ IL-6 ➔ IL-1RA ➔ TGF-β1 ➔
Zebrowska et al. (2015)	Elite cyclists	2.2	Cycling	3 weeks	V02 max ↑

ABBREVIATIONS

AT = Anaerobic threshold, CK = Creatine Kinase, CRP = C-reactive protein, FRAP = Ferric Reducing Antioxidant Power, HITT = High Intensity Intermittent Training, IL-1RA = Interleukin-1 Receptor Antagonist, IL-2 = Interleukin-2, IL-6 = Interleukin-6, MVC = Maximum volume isometric contraction, MDA = Malondialdehyde, ORAC = Oxygen Radical Absorbance Capacity, PGE2 = Prostaglandin E2, TNF-α = Tumour necrosis factor alpha, TGF-β1 = Transforming growth factor beta 1, TTE = Time to exhaustion, V02 max = Maximum aerobic power

LEGEND

↑ - Increased effect

➔ - No change

↓ - Decreased effect

3.3.1 Study Characteristics

The journal of publication was different for each of the studies included. They came from a variety of countries, with two from Iran (Atashak et al. 2013; Ghiasvand et al. 2010), three from the USA (Bloomer et al. 2009; McAnulty et al. 2010; Nieman et al. 2009), one from Australia (Buckley et al. 2009), one from the UK (Jakeman et al. 2017), one from Canada (E. J. H. Lewis et al. 2015), two from Spain (Martorell et al. 2014; Martorell et al. 2015), one from Norway (Raastad et al. 1997), one from Brazil (Santos et al. 2012), one from Denmark (Toft et al. 2000), and one from Poland (Zebrowska et al. 2015).

Further characteristics of the studies are shown in Table 3. All of the randomised controlled trials except one (Toft et al. 2000) used a placebo; this study utilised a non-supplemented control group instead. Measures taken to blind participants and personnel, as well as the outcome, were made in all but three studies (Martorell et al. 2014; Toft et al. 2000; Zebrowska et al. 2015).

One study was intended to be double blind, but should be considered only single blinded due to subjects recognising the distinctive taste of the supplement (Raastad et al. 1997). The remaining studies employed effective double blinding methods.

In addition, two studies used a cross over design with an appropriate washout period (Bloomer et al. 2009; Zebrowska et al. 2015).

Table 3 – Characteristics of studies

Author (year)	Study Type	Number of Participants	Exercise Description	Control Group (number)	Measure of Adherence	Diet and Lifestyle Control
Atashak et al. (2013)	Randomised double blind placebo controlled trial	20	Leg press, leg extension and leg curl resistance exercises at 120% of 1RM (4 sets x 10 repetitions with 3 minutes rest between sets for each exercise)	Same form and size capsule given (10)	Reported by subjects	No omega-3 PUFA or other dietary supplements for 6 months before
Bloomer et al. (2009)	Randomised double blind placebo controlled cross over trial	14	Treadmill walk with weighted backpack (25% of body mass)	(14 – cross over study)	Capsule counting of boxes	Abstinence of alcohol consumption 48 hours preceding test
Buckley et al. (2009)	Randomised double blind placebo controlled trial	25	High intensity sprints, moderate intensity running and football skills training	Sunflower oil (13)	Capsule counting and omega-3 erythrocyte composition	Maintained normal diet under supervision of club dietician
Ghiasvand et al. (2010)	Randomised double blind placebo controlled trial	34	Intensive endurance training	Vitamin E + placebo (8)	None	No antioxidant supplements during and 2 weeks preceding the study

Author (year)	Study Type	Number of Participants	Exercise Description	Control Group (number)	Measure of Adherence	Diet and Lifestyle Control
Jakeman et al. (2017)	Randomised double blind placebo controlled trial	27	10 x 10 repetitions of plyometric drops jumps	Filler oil, flavour masker, gelatine (9)	Supplementation given by researcher not involved in data collection	No recovery methods during the study (ice baths, massages, supplements)
Lewis et al. (2015)	Randomised double blind placebo controlled trial	30	3 squat jumps, 1 minute of pushups, maximum number of reps of 10RM back squats	5ml olive oil with 1000 IU vitamin D (12)	None	<3 servings of fish per week and no omega-3 PUFA supplementation during and 4 weeks prior to the study
Martorell et al. (2014)	Randomised placebo controlled trial	15	Six 2 hour sessions per week, mainly small-sided football games	3.0% almond and 0.8% sucrose, water, flavour, added oils and vitamin E (6)	None described – results included plasma FA levels	Mediterranean diet, 7 day dietary record
Martorell et al. (2015)	Randomised double blind placebo controlled trial	15	Six 2 hour sessions per week, mainly small-sided football games	3.0 wt% almond, water, lemon (40 mg/L) and cinnamon (200 mg/L) flavours, tocopherol acetate (50 mg/L) and 0.8 wt% refined olive oil (6)	None described – results included erythrocyte lipid composition	7 day dietary record

Author (year)	Study Type	Number of Participants	Exercise Description	Control Group (number)	Measure of Adherence	Diet and Lifestyle Control
McAnulty et al. (2010)	Randomised double blind placebo controlled trial	48	3 hours of cycling at approximately 57% maximal wattage	Soybean oil, natural flavors, tocopherols, canola oil, and citric acid (12)	Emailed daily, called weekly for reminders and asked to return empty supplement boxes	No vitamin / mineral supplements, herbs or medications during the study
Nieman et al. (2009)	Randomised double blind placebo controlled trial	23	3 hours of cycling at approximately 57% maximal wattage	Soybean oil, natural flavors, tocopherols, canola oil, and citric acid (12)	None described – plasma EPA and DHA	No vitamin / mineral supplements, herbs or medications during the study
Raastad et al. (1997)	Randomised placebo controlled trial, single blinded (intended to be double blind)	28	Football training – ranging from 6 -7 hours per week averaging 60-85% HR max	650mg corn oil (13)	Plasma omega-3 PUFA composition	Food frequency questionnaire including dietary supplements, such as cod liver oil, fish oil capsules and vitamin supplements

Author (year)	Study Type	Number of Participants	Exercise Description	Control Group (number)	Measure of Adherence	Diet and Lifestyle Control
Santos et al. (2012)	Randomised double blind placebo controlled trial	17	Boot camp with intense physical stress – training included practicing sports, running and weight training	80 mg maltodextrin (8)	Researcher administered capsules during first 3 weeks, and by lieutenant in week 4	24 hour food questionnaire to assess consumption of fatty acids, boot camp involved controlled caloric intake
Toft et al. (2000)	Randomised controlled trial	20	Copenhagen Marathon (18th May 1998)	No placebo given (10)	None	Carbohydrate rich foods (>8 g carbohydrate per kg of body mass per day) and fluids for 6 weeks before
Zebrowska et al. (2015)	Randomised placebo controlled cross over trial	13	Standard cycling test - started with a 3-minute warm-up; intensity increased by 40 Watts every 3 minutes up to maximal intensity	Gelatin capsules (1.3 g lactose monohydrate) (13 – cross over study)	None	No caffeine, antioxidant or alcohol during and 48 hours before

ABBREVIATIONS

1RM = 1 repetition maximum, 10RM = 10 repetition maximum, HR max = Maximum Heart Rate

3.3.2 Participant Demographics

The number of participants ranged from 13 to 48, with an average of 23.5. There was a much smaller variation in the number of participants in the control groups, ranging from six to fourteen, with an average of 10.4. Only one study indicated that the method chosen to determine the sample size was appropriate (Jakeman et al. 2017).

Two studies included female subjects (McAnulty et al. 2010; Nieman et al. 2009), the rest were all male.

Various populations were represented; five studies involved professional athletes, which included Australian rules footballers (Buckley et al. 2009), athletes from summer Olympic sports (E. J. H. Lewis et al. 2015), and professional footballers (Martorell et al. 2014; Martorell et al. 2015; Raastad et al. 1997). A further six groups were trained in a specific sport; handball (Atashak et al. 2013), basketball (Ghiasvand et al. 2010), cycling (McAnulty et al. 2010; Nieman et al. 2009; Zebrowska et al. 2015), and endurance running (Toft et al. 2000). The remaining participants were trained in exercise (Bloomer et al. 2009), physically active (Jakeman et al. 2017), or from the military (Santos et al. 2012).

3.4 Methodology

3.4.1 Eligibility criteria

Within the studies, various conditions that had to be met, such as time spent on training (Bloomer et al. 2009; Jakeman et al. 2017), measure of aerobic power (V02 max) (Bloomer et al. 2009; Raastad et al. 1997), and subjects being non smokers (Bloomer et al. 2009; Martorell et al. 2015; Raastad et al. 1997; Zebrowska et al. 2015). A further four studies ensured that participants were not taking an fish oil supplements before the onset of the experiment (Buckley et al. 2009; E. J. H. Lewis et al. 2015; Raastad et al. 1997; Zebrowska et al. 2015), of which two also put a limit on the number of portions of fish

eaten per week (Buckley et al. 2009; E. J. H. Lewis et al. 2015). Only six studies explicitly stated that subjects were healthy (Atashak et al. 2013; Bloomer et al. 2009; Ghiasvand et al. 2010; Jakeman et al. 2017; Raastad et al. 1997; Zebrowska et al. 2015), although this was included for the eligibility criteria for this review. Three studies did not state the eligibility criteria for subjects (Martorell et al. 2014; McAnulty et al. 2010; Nieman et al. 2009).

3.4.2 Duration of supplementation

All but one study (Jakeman et al. 2017), supplemented participants with omega-3 over a number of weeks. Jakeman et al. (2017) gave a one-off acute dose post exercise. The period of supplementation in the other studies ranged from one week (Atashak et al. 2013) to ten weeks (Raastad et al. 1997); although due to the nature of testing and cross over design of some studies, there were further variations in the total study time. Two studies supplemented for three weeks (E. J. H. Lewis et al. 2015; Zebrowska et al. 2015), one for four weeks (Santos et al. 2012), one for five weeks (Buckley et al. 2009), five for six weeks (Bloomer et al. 2009; Ghiasvand et al. 2010; McAnulty et al. 2010; Nieman et al. 2009; Toft et al. 2000), and two for eight weeks (Martorell et al. 2014; Martorell et al. 2015).

3.4.3 Omega-3 PUFA supplementation

Only three studies did not use a combination of both EPA and DHA. One used just EPA (Ghiasvand et al. 2010) and two others used only DHA (Martorell et al. 2014; Martorell et al. 2015).

There was much variation in the dose range of each omega-3 PUFA, for EPA it was between 0.150 g/day up to 2.224 g/day, and for DHA it was between 0.100 g/day to 2.208 g/day. The lowest doses were from Jakeman et al. (2017), and the highest doses

from Bloomer et al. (2009).

Half of the studies included other types of omega-3 PUFA apart from EPA and DHA; such as ALA (Atashak et al. 2013; Lewis et al. 2015; Martorell et al. 2015; Raastad et al. 1997; Toft et al. 2000; Zebrowska et al. 2015) and docosapentaenoic acid (DPA) (Martorell et al. 2014). Six of the studies had vitamin E added to the supplement (Ghiasvand et al. 2010; Martorell et al. 2014; Martorell et al. 2015; McAnulty et al. 2010; Toft et al. 2000; Zebrowska et al. 2015). Only two studies supplemented exclusively with EPA and DHA with no other additives (Bloomer et al. 2009; Buckley et al. 2009).

Three studies did not provide the supplementation in capsule form; opting for liquid seal oil (E. J. H. Lewis et al. 2015) or a one litre beverage containing different oils (Martorell et al. 2014; Martorell et al. 2015).

There was significant heterogeneity across the studies in the way they implemented their supplementation protocol. In two of the studies the supplementation protocol was not described (Ghiasvand et al. 2010; Toft et al. 2000), in two others the supplements were administered by personnel involved in the study (Jakeman et al. 2017; Santos et al. 2012), and the remainder relied on participants to take the supplements themselves.

3.4.4 Measure of adherence

One study relied on adherence to be reported by subjects (Atashak et al. 2013), two utilised a form of capsule counting (Bloomer et al. 2009; McAnulty et al. 2010), one used capsule counting in combination with the erythrocyte omega-3 composition (Buckley et

al. 2009), two administered the supplements using personnel involved in the research (Jakeman et al. 2017; Santos et al. 2012), and one used plasma omega-3 composition (Raastad et al. 1997). The remaining studies did not state a measure of adherence (Ghiasvand et al. 2010; E. J. H. Lewis et al. 2015; Toft et al. 2000; Zebrowska et al. 2015). However, the results of three studies included either erythrocyte fatty acid (Martorell et al. 2015) or plasma fatty acid composition (Martorell et al. 2014; Nieman et al. 2009); as they were used as measures of compliance elsewhere (Buckley et al. 2009; Raastad et al. 1997), they can similarly be considered here.

3.4.5 Type of exercise

There was considerable diversity in the exercise protocol chosen in the studies; one used leg resistance exercises (Atashak et al. 2013), two involved running, one on a treadmill with a weighted backpack (Bloomer et al. 2009), and the other was after the Copenhagen Marathon in 1998 (Toft et al. 2000). Other exercises included a mixed training programme (Buckley et al. 2009; E. J. H. Lewis et al. 2015), "intensive endurance training" (Ghiasvand et al. 2010), plyometric drop jumps (Jakeman et al. 2017), football training (Martorell et al. 2014; Martorell et al. 2015; Raastad et al. 1997), cycling (McAnulty et al. 2010; Nieman et al. 2009; Zebrowska et al. 2015) and a military boot camp (Santos et al. 2012).

3.4.6 Control factors: Diet and lifestyle

Twelve studies monitored the subjects food intake and diet over the courses of the study, whereas the two studies that did not used other methods (Ghiasvand et al. 2010; Jakeman et al. 2017). Six studies put a restriction on physical activity preceding their outcome testing (Atashak et al. 2013; Bloomer et al. 2009; Jakeman et al. 2017; E. J. H. Lewis et al. 2015; Toft et al. 2000; Zebrowska et al. 2015), four restricted any form of nutritional

supplementation (including omega-3) (Atashak et al. 2013; Jakeman et al. 2017; McAnulty et al. 2010; Nieman et al. 2009), two instructed abstinence of alcohol consumption (Bloomer et al. 2009; Zebrowska et al. 2015), and two prohibited antioxidant supplementation (Ghiasvand et al. 2010; Zebrowska et al. 2015).

3.5 The effect of omega-3 supplementation on recovery from exercise

3.5.1 Inflammation

Eight of the studies measured inflammation to assess whether omega-3 had any benefits (Atashak et al. 2013; Bloomer et al. 2009; Ghiasvand et al. 2010; Jakeman et al. 2017; Martorell et al. 2014; Nieman et al. 2009; Santos et al. 2012; Toft et al. 2000). Of these, five measured C-reactive protein (CRP) (Atashak et al. 2013; Bloomer et al. 2009; Martorell et al. 2014; Nieman et al. 2009; Santos et al. 2012); five measured various cytokines such as tumour necrosis factor alpha (TNF-α) (Bloomer et al. 2009), interleukin-2 (IL-2) and TNF-α (Ghiasvand et al. 2010), interleukin-6 (IL-6) (Jakeman et al. 2017), interleukin-1 receptor antagonist (IL-1RA), IL-6 and interleukin-8 (IL-8) (Nieman et al. 2009), and TNF-α, IL-6, IL-1RA and transforming growth factor beta 1 (TGF-β1) (Toft et al. 2000); and one included prostaglandin E2 (PGE2) (Martorell et al. 2014).

Four of the studies found a statistically significant effect of omega-3 on inflammation after exercise (Atashak et al. 2013; Bloomer et al. 2009; Martorell et al. 2014; Santos et al. 2012), whereas the other four that measured inflammation did not (Ghiasvand et al. 2010; Jakeman et al. 2017; Nieman et al. 2009; Toft et al. 2000). Only one of the studies that measured CRP did not find a significant effect (Nieman et al. 2009).

3.5.2 Oxidative stress

Five studies used malondialdehyde (MDA) as a measure oxidative stress (Atashak et al. 2013; Bloomer et al. 2009; Ghiasvand et al. 2010; Martorell et al. 2014; Martorell et al. 2015), and one used F_2-isoprostanes (McAnulty et al. 2010). Of the five studies that used MDA as a measure of oxidative stress, two found a statistical difference (Atashak et al. 2013; Ghiasvand et al. 2010). McAnulty (2010) also found a significant difference, but used a difference measure as described above. The three other studies that did not find an association between omega-3 and oxidative stress all included a measure of MDA, as well as other markers of protein modification and nitrosative damage (Bloomer et al. 2009; Martorell et al. 2014; Martorell et al. 2015).

3.5.3 Antioxidant status

Four studies measured antioxidant status, using erythrocyte antioxidant enzymes such as catalase (Ghiasvand et al. 2010; Martorell et al. 2014; Martorell et al. 2015) and other measures, including ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) (McAnulty et al. 2010). One study found a significant effect on antioxidant enzyme status (Martorell et al. 2015).

3.5.4 Cellular / muscle damage

Of the studies that used a measure of cellular or muscle damage, all six used creatine kinase (CK) (Atashak et al. 2013; Bloomer et al. 2009; Jakeman et al. 2017; Martorell et al. 2014; Santos et al. 2012; Toft et al. 2000). Of which, one included a perceptual measure of damage (Jakeman et al. 2017), one used lactate dehydrogenase (LDH) (Atashak et al. 2013) and another used LDH, aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyl transpeptidase (ϒGT) (Martorell et al. 2014). One study found

omega-3 PUFA supplementation attenuated the rise in CK (Atashak et al. 2013). None of the other studies listed above that used measures of cellular or muscle damage reported a significant reduction with omega-3 supplementation.

3.5.5 Exercise performance

Six studies measured exercise performance before and after supplementation (Buckley et al. 2009; Jakeman et al. 2017; E. J. H. Lewis et al. 2015; Nieman et al. 2009; Raastad et al. 1997; Zebrowska et al. 2015), of which two measured maximal aerobic capacity (V02 max) (Nieman et al. 2009; Zebrowska et al. 2015), two used V02 max and time to exhaustion (TTE) (Buckley et al. 2009; Raastad et al. 1997), and two used measures of muscle strength and activation (Jakeman et al. 2017; E. J. H. Lewis et al. 2015). Two studies found an improvement in muscle strength and activation after omega-3 supplementation (Jakeman et al. 2017; E. J. H. Lewis et al. 2015). Of the studies that measured aerobic power, one found an increase in V02 max (Zebrowska et al. 2015).

4 DISCUSSION

The aim of this review was to assess the current evidence in relation to the impact of omega-3 polyunsaturated fatty acids on recovery from exercise in trained males.

4.1 Study design and participant demographics

All the studies included were randomised controlled trials (RCTs), which are considered the gold standard when comparing one treatment to another, ranking highly in the hierarchy of evidence when trying to determine effectiveness (Sackett et al. 1996). The widespread use of double blinding in ten of the fourteen studies further enhances their reliability. Although, the one study that only used single blinding methods (Raastad et al. 1997) found no effect on any outcomes measured. The fact that the only study to not use a placebo (Toft et al. 2000) also found no effect highlights the importance of ensuring adequate study design to produce reliable results.

Despite selection criteria ensuring participants were comparable, there remains some heterogeneity between the demographics. Confounding factors such as ethnicity, age, sporting background, and professional level within their respective sports can impact the quality of the studies if researchers do not account for them. Even within studies this presented problems. For example, Lewis et al. (2015) found athletes from different sports had variability in the endurance and strengths aspects of exercise testing. This is particular importance considering that the only primary outcome of this study was a change in exercise performance.

4.2 Sample size

Determining sample size is an important primary step when conducting a RCT (Noordzij et al. 2010). Small sample sizes can over estimate the size of effects or leading to incorrect

results. Nevertheless, only Jakeman (2017) used a calculation of statistical power to ensure an adequate sample size, meaning the 'power [was] at or above 80%'. The two studies (Bloomer et al. 2009; Zebrowska et al. 2015) that had the lowest number of total participants (14 and 13 respectively) both employed a cross over design. This enabled both the supplementation and control groups to contain equal numbers of total participants, and furthermore reduces heterogeneity between the participant demographics. Studies by Martorell (2014; 2015) used a relatively small sample size. Despite having nearly identical study designs in all aspects apart from outcomes measured, they produced contrasting results with regards to inflammation and antioxidant effect. Although Raastad (1997) used a slightly larger sample size, it was not sufficient enough to detect a change in V02 max below 2 ml / (kg . minute). Therefore, sample size may be a contributing factor as to why this study did not find an effect of supplementation.

4.3 Type of exercise

4.3.1 Resistance exercise

The studies selected used a variety of exercises. Clearly the impact of omega-3 PUFA on the recovery of exercise requires a sufficiently damaging procedure in order to see an effect. Resistance training is known to produce EIMD, resulting in localised inflammation (Proske & Morgan 2001) due to the eccentric movement. Studies that employed a weightlifting training protocol all successfully induced inflammation, as shown by a significant increase in CRP compared to pre-exercise levels (Atashak et al. 2013; Bloomer et al. 2009; Santos et al. 2012). Furthermore, statistical analysis showed a reduction in CRP in each of the supplementation groups compared to the placebo, suggesting that omega-3 PUFAs have potential benefits in reducing inflammation after resistance

exercise. This was the only mode of exercise where every study found a significant effect of omega-3 PUFAs on at least one of the outcome measures. However, within studies where resistance exercise was employed, there were discernible differences. This manifested itself as differences in the actual weightlifting exercise used (including the use of free weights versus machines), mass of the weights used, and the number of sets and repetitions performed; all of which are integral factors that must be controlled when attempting to induce EIMD.

4.3.2 Cycling

Other types of exercises had greater amounts of heterogeneity. Cycling is considered to be a primarily aerobic exercise and therefore, tends to produce different effects to resistance exercise. The available data demonstrates a mixed effect of omega 3 on cycling depending on which outcome measure is used. Nieman et al. (2009) and McAnulty et al. (2010), shared the same cycling protocol due to shared authorship. Though direct comparison is difficult because of differing outcome measures. McAnulty et al. (2010) did find beneficial effects on oxidative stress, however as no other cycling studies investigated this, it cannot be considered a truly reliable conclusion. This is a common theme throughout studies using cycling protocols.

Furthermore, supplementation did not yield any benefits in relation to measures of antioxidant status, inflammation, and exercise performance in any cycling studies. Notwithstanding the clear benefits in these three measures, when alternative exercises were used. Once again outcomes were measured in individual cycling studies. Despite McAnulty et al. (2010) using two distinct antioxidant measurements (FRAP and ORAC), no effect was found. Nieman et al. (2009) was the only study not to show a reduction in

inflammation; suggesting that the endurance cycling protocol used was not sufficient enough to induce EIMD and the associated increase in inflammation.

The aerobically trained nature of participants may account for the lack of improvement of time trial. In addition other studies that used endurance training in combination with an alternate exercise modality (Raastad et al. 1997; Toft et al. 2000), did not find an enhancement in performance. Another possible explanation is that the cycling tests were not intensive enough. Zebrowska et al. (2015) used a very similar cycling protocol to the one used in the two other studies; where there were incremental increases in wattage over time, but a larger increase per minute. This may explain why it was the only cycling study to show an improvement in aerobic performance.

4.3.3 Running

The most common mode of exercise used was running. However this was also the group within which there was the greatest disparity between outcomes, as well as over half the studies showing no effect (Buckley et al. 2009; Raastad et al. 1997; Toft et al. 2000). These studies all had significant limitation. No studies described a supplementation protocol, or measured adherence. Such flaws in the study design make it more problematic when ascertaining whether it was the exercise choice that impacted the outcomes.

4.4 Type of test used

4.4.1 Inflammation

The evidence of an impact of omega-3 PUFAs on inflammation has mixed results. Studies using CRP were more likely to show an attenuation of inflammation using supplementation. Santos et al. (2012) utilised an ultra-sensitive measure of CRP and found a significant effect; where as studies that did not include CRP showed no effect. Though this may be due to the over-specificity of the alternative inflammatory

biomarkers used. However, CRP is an acute phase protein known to follow a rise in IL-6, therefore one may expect an equal effect on both. It is possible that studies that did not find an effect on IL-6 did not produce a sufficiently damaging exercise protocol to induce systemic inflammation (Jakeman et al. 2017; Nieman et al. 2009; Toft et al. 2000). The anti-inflammatory effects of omega-3 PUFA may be due to a direct reduction in CRP, inflammatory cytokines, or both (Calder 2006). When comparing types of exercise it is clear that resistance exercise is more likely to produce and effect than endurance or aerobic activity.

4.4.2 Oxidative stress

The data in relation to oxidative stress is inconclusive. The double bonds within omega-3 PUFAs are susceptible to lipid peroxidation and therefore theoretically influence increases in oxidative stress. However, production of certain reactive species from oxidative stress does have beneficial effects on skeletal muscle in response to exercise (Barbieri & Sestili 2012), suggesting there is an optimal amount of oxidative species. It has been suggested that because omega-3 PUFAs are tightly packed in membranes, the double bonds are less exposed to oxidation (Mori et al. 1999). There was also variability depending on the markers used. The majority of the studies used MDA however this outcome produced dissimilar results. McAnulty et al. (2010) was the only study overall to use F2- isoprostanes; considered to be the gold standard when determining oxidative stress (Milne et al. 2005). The strength of this measure could arguably be diminished by the fact that both the supplementation and placebo groups received known antioxidants such as vitamins C, E and A. It is unclear which exercise types and markers would be affected by supplementation. Even if more definitive answers could be found, the adaptive benefits of a reduction of oxidative stress on recovery from exercise are ambiguous.

4.4.3 Antioxidant activity

It is unlikely that there was a significant effect on antioxidant activity with only one paper finding an effect. Disagreement between the effects of omega-3 PUFA supplementation is most apparent when contrasting two studies by Martorell et al. (2014; 2015). Despite using identical study designs, outcomes varied. Of these studies only one found an effect on antioxidant activity even though the same measure was used. This weakens the conclusions from these studies regarding antioxidant status, and arguably the studies as a whole. It would be expected identical studies produce reliable results. Furthermore, even when multiple antioxidants were measured within studies, no effect was found.

4.4.4 Cellular / muscle damage

Studies measuring the effect of exercise on muscle damage all found a significant effect in association to time. However, only Atashak et al. (2013) found that omega-3 PUFA supplementation attenuated a rise in CK. This may be because it employed resistance exercise in which the eccentric portion is known to induce myocellular damage; none of the other studies that measured CK used this method. Various factors can cause a rise in CK of which is a high muscle mass. Participants of all studies would be likely to have a higher muscle mass than the general population, explaining why the CK levels in the studies remained elevated. Furthermore, in one of the studies (Santos et al. 2012), the standard deviations for CK measurements were almost equal to the mean values, highlighting considering individual variability.

4.4.5 Exercise performance

The potential benefits of omega-3 PUFAs on exercise performance stem from two different mechanisms; alterations in the cardiovascular response after exercise and an

amelioration of EIMD. Studies that found an increase in exercise performance reported decreased functional and perceptual measures of EIMD (Jakeman et al. 2017; E. J. H. Lewis et al. 2015). This was mainly through reduced delayed onset muscle soreness (DOMS), allowing faster recovery in performance ability. Jakeman et al. (2017) was the only study to supplement with both high and low dose omega-3 PUFAs. To ensure that the results had some practical applicability, due to the relatively small sample size, both statistical significance and effect sizes were considered. This confirmed that the high dose group demonstrated a smaller decrease in capability compared to both the low dose and placebo groups.

Evidence from the studies included suggests omega-3 PUFAs have no impact on endurance performance. This may be because the cardiovascular benefits seen in untrained populations do not apply to aerobically trained individuals, due to their altered baselines for cardiovascular performance factors, such as cardiac output and peripheral blood flow.

4.5 Omega-3 PUFA supplementation

4.5.1 Dosage

There appears to be no association between the dosage of EPA and DHA used and the effect on recovery. Although, due to the complexity of the included studies it is possible that this review was underpowered for statistical analysis of effect. Consequently, in order to calculate the magnitude of effect in relation to dosage, a meta-analysis would likely be required. Interpretation of effect is made more problematic by the fact that the dose range of EPA and DHA per day was similar between all studies. Only Lewis et al. (2015) justified their dosage; arguing that other studies have used “super-physiological doses”, which may not be tolerable, or too expensive to take over a sustained period. The

use of realistic dosages in these studies means that the conclusions have greater relevance because they can easily be put into practice. Any differences in dose may have been offset by the accumulation of omega-3 PUFAs over the difference lengths of treatment period. Pre-intervention control of omega-3 PUFA intake may also account for the limited of correlation. Perhaps surprisingly, the studies that showed no effect on any outcomes used some of the highest doses, indicating other factors have a greater influence on the efficacy of treatment.

4.5.2 Duration of supplementation

Outcomes do not appear to be affected by the length of the supplementation period. Interestingly, studies showing an improvement in exercise performance had the shortest duration of supplementation. It is thought that gradual incorporation of omega-3 PUFAs into cell membranes is responsible for their effects. However, Jakeman et al. (2017) demonstrated that even an acute one-off dose of EPA and DHA has a beneficial impact between one up to 96 hours after exercise, suggesting alternative modes of action during the acute response. It is worth noting that this acute dose was significantly smaller than that used in other studies, including those finding no effects. Furthermore, studies that did not show an improvement in exercise performance used considerably longer supplementation periods (Nieman et al. 2009; Raastad et al. 1997; Toft et al. 2000). These findings indicate that acute or short-term supplementation may be more beneficial; especially to athletes during intensive training camps or competitions spanning several days. This is due to the beneficial effects of EIMD and inflammation being superseded by the desire to maintain peak performance.

4.6 Control of diet and omega-3 PUFA intake

The studies suggest that omega-3 PUFA supplementation and dietary intervention are important confounding factors, and failure to control them would lead to unreliable outcomes. In studies that did not restrict supplementation before their own intervention found no effect on oxidative stress or muscle damage. Whereas studies that restricted supplementation outside the protocol showed an effect when their own omega-3 PUFA supplementation was implemented. In addition, all studies that showed improved outcomes on exercise performance limited supplementation, including omega-3 PUFAs, before their trial.

4.7 Strengths

This review has several strengths. An extremely thorough search strategy was conducted to ensure careful selection of literature. The Boolean term 'NOT' was omitted from searches, which avoided early elimination of potentially useful studies. Multiple databases were assessed but were not included as there was not any gain in additional references. Deduplication of references is an integral part of systematic reviews (Moher et al. 2009). A systematic review by Kwon et al (2015) found that there is no singular preferred option for deduplication. In order to minimise the number of false negatives and false positives both Mendeley and Endnote were used. Despite this, there remains some discrepancy in automated methods of deduplication. Papers were also hand searched accordingly for false positives before deleting both internal and external duplicates; combination of these methods yields the best results (Qi et al. 2013). Covidence, which is the standard production platform for Cochrane reviews, was used for the title and abstract screen. Use of the CONSORT checklist allowed for a more structured and in depth appraisal than if no tool had been used.

4.8 Limitations of this review

4.8.1 Identification and selection of studies

As with all reviews, there is a possibility of publication bias, whereby valid studies that showed negative results were not published. Journals tend to publish papers that show a positive effect, meaning some papers could have been overlooked. Despite the aforementioned process undertaken to remove duplicates, there is no predefined method for doing so. Access to certain papers was also limited due to the funding available for this review. Though measures were undertaken in an attempt to retrieve these papers, it was not feasible.

4.8.2 Heterogeneity of papers

The role of omega-3 PUFAs in the field of sports and exercise science has only been explored recently. There is a relatively small numbers of studies overall, hence an even smaller amount for each exercise type. The implementation of different exercise protocols makes overall comparison more problematic. This issue also arises when comparing the different techniques used when measuring the same outcomes. Meta-analysis were not performed because of the heterogeneity of the study design, exercise type, difference in outcomes, and variation in omega-3 PUFA supplementation.

4.8.3 Quality assessment

Although the quality of papers was assessed, there is no 'gold standard' for critical appraisal tools (Katrak et al. 2004). This allowed the inclusion of papers that met the criteria, but had limited usefulness when attempting to answer the review question. The most notable example of which is the study by Ghiasvand et al. (2010), from which limited conclusions can be drawn. The CONSORT checklist does not result in a score, and it is possible that this paper may have been excluded if a scoring system was implemented.

4.8.4 Implications for future

Whilst ten out of the fourteen included studies reported a statistically significant benefit in the various outcome measures used; methodological issues used within the studies limit the validity of the conclusions that can be drawn. Comparisons across studies are also problematic due to the heterogeneity in the study designs. There was also some disagreement amongst similar outcome measures. The conclusions drawn from this review have been suggested elsewhere, many within the papers reviewed. Although there is not enough information on which to base clinical practice; there may be enough for people that exercise to take the results into consideration when choosing whether to take omega-3 PUFA supplements. Grey literature, particularly online, strongly encourages supplementation with omega-3 PUFAs in certain sports such as bodybuilding. In this way it is apparent that current research is influencing opinions and patient practice rather than guidelines and clinical outcomes.

Future studies should focus on resistance exercise using athletes with similar abilities undertaking high intensity exercises, allowing focus on one energy system. Longer supplementation protocols would likely lead to more applicable results, as acute supplementation of omega-3 PUFAs is rarely practiced. Depending on the outcomes measured, a variety of markers should be used. Finally, dosage should be considered in relation to body mass and different ratios of EPA: DHA should be used.

5 CONCLUSION

Recent literature already recommends using omega-3 PUFA supplementation for general athletic populations. Whilst it is clear that there are widespread beneficial effects of supplementation, determination of said effect is largely dependant on outcome measures. Omega-3 PUFAs are known to reduce inflammation and therefore the exercise implemented must be extensive enough to cause inflammation, induce muscle injury, or induce oxidative stress. The response to exercise is largely influenced by the prior training an athlete has received. Therefore, the difference in sporting ability is reflected in the results. From a practical perspective, improvements seen in performance will be of the greatest interest to athletes, though the precise mechanisms need further investigation. However, due to poor sample sizes and the lack of replication the size of this effect is difficult to determine. Variation across studies in both duration and dosage of omega-3 PUFAs has yielded different results. In addition, whilst the dose ratio of EPA: DHA has been thoroughly explored in relation to cardiovascular disease, the same cannot be said for exercise. As such, future studies should focus on increasing the validity of studies already available and investigating an optimal supplementation strategy.

REFERENCES

Aslan, A. & Triadafilopoulos, G., 1992. Fish oil fatty acid supplementation in active ulcerative colitis: a double-blind, placebo-controlled, crossover study. *The American journal of gastroenterology*, 87(4), pp.432–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1553930 [Accessed May 25, 2017].

Atashak, S. et al., 2013. Effect of omega-3 supplementation on the blood levels of oxidative stress, muscle damage and inflammation markers after acute resistance exercise in young athletes. *Kinesiology*, 45(1), pp.22–29. Available at: http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2013-27207-003&site=ehost-live&scope=site%5Cnatashak_sirvan@yahoo.com.

Bang, H.O., Dyerberg, J. & Nielsen, A., 1971. PLASMA LIPID AND LIPOPROTEIN PATTERN IN GREENLANDIC WEST-COAST ESKIMOS. *The Lancet*, 297(7710), pp.1143–1146. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0140673671916588 [Accessed April 6, 2017].

Barbieri, E. & Sestili, P., 2012. Reactive oxygen species in skeletal muscle signaling. *Journal of signal transduction*, 2012, p.982794. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22175016 [Accessed May 28, 2017].

Bloomer, R.J. et al., 2009. Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: a randomized, placebo controlled, cross-over study. *Lipids in Health and Disease*. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2735747/pdf/1476-511X-8-36.pdf [Accessed April 11, 2017].

Bucher, H.C. et al., 2002. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *The American journal of medicine*, 112(4), pp.298–304. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11893369 [Accessed March 30, 2017].

Buckley, J.D. et al., 2009. DHA-rich fish oil lowers heart rate during submaximal exercise in elite Australian Rules footballers. *Journal of Science and Medicine in Sport*, 12, pp.503–507. Available at: www.sciencedirect.com [Accessed April 8, 2017].

Calder, P.C., 2006. N-3 Polyunsaturated Fatty Acids, Inflammation, and Inflammatory Diseases. *The American journal of clinical nutrition*, 83(6 Suppl), p.1505S–1519S. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16841861.

Dyerberg, J. et al., 1978. EICOSAPENTAENOIC ACID AND PREVENTION OF THROMBOSIS AND ATHEROSCLEROSIS? Available at: http://ac.els-cdn.com/S0140673678915052/1-s2.0-S0140673678915052-main.pdf?_tid=641b863a-1ab7-11e7-8660-00000aacb362&acdnat=1491476242_61b410d04780d87392a12f76fd825319 [Accessed April 6, 2017].

Fortin, P.R. et al., 1995. Validation of a meta-analysis: the effects of fish oil in rheumatoid arthritis. *Journal of clinical epidemiology*, 48(11), pp.1379–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7490601 [Accessed May 25, 2017].

Freeman, M.P., 2009. Omega-3 Fatty Acids in Major Depressive Disorder. *The Journal of Clinical Psychiatry*, 70(suppl 5), pp.7–11. Available at: http://article.psychiatrist.com/?ContentType=START&ID=10006547 [Accessed April 1, 2017].

Fritsche, K., 2006. Fatty Acids as Modulators of the Immune Response. *Annual Review of Nutrition*, 26(1), pp.45–73. Available at: http://www.annualreviews.org/doi/10.1146/annurev.nutr.25.050304.092610 [Accessed May 25, 2017].

Ghiasvand, R. et al., 2010. Effect of Eicosapentaenoic Acid (EPA) and Vitamin E on the Blood Levels of Inflammatory Markers, Antioxidant Enzymes, and Lipid Peroxidation in Iranian Basketball Players. *Iranian J Publ Health*, 39(1), pp.15–21. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3468972/pdf/ijph-39-015.pdf [Accessed April 10, 2017].

GISSI, 1999. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of theGISSI-Prevenzione trial. *The Lancet*, 354(9177), pp.447–455. Available at: http://www.sciencedirect.com/science/article/B6T1B-3X99PCK-8/2/354d89e01e475493786f2a0f9dc333c0 [Accessed March 31, 2017].

Gray, P. et al., 2014. Fish Oil Supplementation Reduces Markers of Oxidative Stress but Not Muscle Soreness after Eccentric Exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 24(2), pp.206–214. Available at: http://journals.humankinetics.com/doi/10.1123/ijsnem.2013-0081 [Accessed April 3, 2017].

Helland, I.B. et al., 2003. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics*, 111(1), pp.e39-44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12509593 [Accessed May 25, 2017].

Hingley, L. et al., 2017. DHA-Rich Fish Oil Increases the Omega-3 Index and Lowers the Oxygen Cost of Physiologically Stressful Cycling in Trained Individuals. *International Journal of Sport Nutrition and Exercise Metabolism*, pp.1–28. Available at: http://journals.humankinetics.com/doi/10.1123/ijsnem.2016-0150 [Accessed April 3, 2017].

Jakeman, J.R. et al., 2017. Effect of an acute dose of omega-3 fish oil following exercise-induced muscle damage. *European Journal of Applied Physiology*, pp.1–8. Available at: http://link.springer.com/10.1007/s00421-017-3543-y [Accessed April 2, 2017].

Katrak, P. et al., 2004. A systematic review of the content of critical appraisal tools. *BMC medical research methodology*, 4, p.22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15369598 [Accessed May 29, 2017].

Kris-Etherton, P.M., Harris, W.S. & Appel, L.J., 2002. Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Circulation*, 106(21). Available at: http://circ.ahajournals.org/content/106/21/2747.short [Accessed March 28, 2017].

Lewis, E. et al., 2015. 21 Days of Mammalian Omega-3 Fatty Acid Supplementation Improves Aspects of Neuromuscular Function and Performance in Male Athletes Compared To Olive Oil Placebo. *Journal of the International Society of Sports Nutrition*, 12(1), p.28. Available at: http://www.jissn.com/content/12/1/28 [Accessed April 10, 2017].

Li, K. et al., 2014. Effect of Marine-Derived n-3 Polyunsaturated Fatty Acids on C-Reactive Protein, Interleukin 6 and Tumor Necrosis Factor α: A Meta-Analysis W.-H. Schunck,

ed. *PLoS ONE*, 9(2), p.e88103. Available at: http://dx.plos.org/10.1371/journal.pone.0088103 [Accessed April 12, 2017].

Lorenz, D. & Reiman, M., 2011. The role and implementation of eccentric training in athletic rehabilitation: tendinopathy, hamstring strains, and acl reconstruction. *International journal of sports physical therapy*, 6(1), pp.27–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21655455 [Accessed May 25, 2017].

Margaritelis, N. V. et al., 2015. Muscle damage and inflammation after eccentric exercise: can the repeated bout effect be removed? *Physiological Reports*, 3(12). Available at: http://physreports.physiology.org/content/3/12/e12648 [Accessed May 27, 2017].

Martorell, M. et al., 2015. Docosahexaenoic Acid Supplementation Promotes Erythrocyte Antioxidant Defense and Reduces Protein Nitrosative Damage in Male Athletes. *Lipids*, 50, pp.131–148. Available at: http://download.springer.com/static/pdf/942/art%253A10.1007%252Fs11745-014-3976-6.pdf?originUrl=http%3A%2F%2Flink.springer.com%2Farticle%2F10.1007%2Fs11745-014-3976-6&token2=exp=1491823986~acl=%2Fstatic%2Fpdf%2F942%2Fart%25253A10.1007%25252Fs11745-014-397 [Accessed April 10, 2017].

Martorell, M. et al., 2014. Effect of DHA on plasma fatty acid availability and oxidative stress during training season and football exercise. *Food & function*, 5(8), pp.1920–1931. Available at: http://pubs.rsc.org/en/content/articlepdf/2014/fo/c4fo00229f [Accessed April 10, 2017].

McAnulty, S.R. et al., 2010. Effect of n-3 Fatty Acids and Antioxidants on Oxidative Stress after Exercise. *Medical Science Sports Exercise*, 42(9), pp.1704–1711. Available at: http://ovidsp.uk.ovid.com/sp-3.9.1a/ovidweb.cgi?&S=NGODPDNEHLHFNOPGFNNKHHEGDOHFAA00&Link+Set=S.sh.46%7C6%7Csl_190 [Accessed April 10, 2017].

Milne, G.L., Musiek, E.S. & Morrow, J.D., 2005. F2-Isoprostanes as markers of oxidative stress in vivo: An overview. *Biomarkers*, 10(sup1), pp.10–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16298907 [Accessed May 27, 2017].

Moher, D. et al., 2009. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Medicine*, 6(7), p.e1000097. Available at: http://dx.plos.org/10.1371/journal.pmed.1000097 [Accessed April 12, 2017].

Mori, T.A. et al., 1999. Effect of dietary fish and exercise training on urinary F2-isoprostane excretion in non-insulin-dependent diabetic patients. *Metabolism: clinical and experimental*, 48(11), pp.1402–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10582548 [Accessed May 28, 2017].

NICE, 2008. Lipid modification: cardiovascular risk assessment and the modification of blood lipids for the primary and secondary prevention of cardiovascular disease | Guidance and guidelines | NICE. Available at: https://www.nice.org.uk/guidance/cg181 [Accessed May 25, 2017].

Nieman, D.C. et al., 2009. n-3 Polyunsaturated Fatty Acids Do Not Alter Immune and Inflammation Measures in Endurance Athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, 19(5), pp.536–546.

Niu, K. et al., 2006. Dietary long-chain n-3 fatty acids of marine origin and serum C-reactive protein concentrations are associated in a population with a diet rich in marine products. *The American journal of clinical nutrition*, 84(1), pp.223–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16825699 [Accessed May 22, 2017].

Noordzij, M. et al., 2010. Sample size calculations: basic principles and common pitfalls. *Nephrology Dialysis Transplantation*, 25(5), pp.1388–1393. Available at: https://academic.oup.com/ndt/article-lookup/doi/10.1093/ndt/gfp732 [Accessed May 23, 2017].

Oliver, C. & Watson, H., 2016. Omega-3 fatty acids for cystic fibrosis. In H. Watson, ed. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd. Available at: http://doi.wiley.com/10.1002/14651858.CD002201.pub5 [Accessed May 27, 2017].

Proske, U. & Morgan, D.L., 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of physiology*, 537(Pt 2), pp.333–45. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/11731568 [Accessed May 27, 2017].

Qi, X. et al., 2013. Find Duplicates among the PubMed, EMBASE, and Cochrane Library Databases in Systematic Review. *PLoS ONE*, 8(8). Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3748039/pdf/pone.0071838.pdf [Accessed April 7, 2017].

Raastad, T., Høstmark, a T. & Strømme, S.B., 1997. Omega-3 fatty acid supplementation does not improve maximal aerobic power, anaerobic threshold and running performance in well-trained soccer players. *Scandinavian journal of medicine & science in sports*, 7(1), pp.25–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9089901.

Sackett, D.L. et al., 1996. Evidence based medicine: what it is and what it isn't. *BMJ*, 312(7023). Available at: http://www.bmj.com/content/312/7023/71 [Accessed May 23, 2017].

Santos, E.P. et al., 2012. The production of C-reactive protein in military personnel during 5 days of intense physical stress and nutritional restriction. *Biology of Sport*, 29(2), pp.93–99.

Schulz, K.F. et al., 2010. CONSORT 2010 Statement CONSORT 2010 Statement : updated guidelines for reporting parallel group randomised trials. *Development*, 1(2), pp.1–6. Available at: http://www.consort-statement.org/Media/Default/Downloads/CONSORT 2010 Statement/CONSORT 2010 Statement - Journal of Clinical Epidemiology.pdf [Accessed April 14, 2017].

Simmer, K., Patole, S.K. & Rao, S.C., 2011. Longchain polyunsaturated fatty acid supplementation in infants born at term. In K. Simmer, ed. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd. Available at: http://doi.wiley.com/10.1002/14651858.CD000376.pub3 [Accessed May 25, 2017].

Simmer, K., Schulzke, S. & Patole, S., 2008. Longchain polyunsaturated fatty acid supplementation in preterm infants. In K. Simmer, ed. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd. Available at: http://doi.wiley.com/10.1002/14651858.CD000375.pub3 [Accessed May 25, 2017].

Simopoulos, A., 2008. The Importance of the Omega-6 / Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. *Experimental Biology and Medicine*, 233(6), pp.674–688. Available at: http://journals.sagepub.com/doi/pdf/10.3181/0711-MR-311 [Accessed March 30, 2017].

Simopoulos, A.P., 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition*, 21(6), pp.495–505. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12480795 [Accessed April 10, 2017].

Starbuck, C. & Eston, R.G., 2012. Exercise-induced muscle damage and the repeated bout effect: evidence for cross transfer. *European Journal of Applied Physiology*, 112(3), pp.1005–1013. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21720885 [Accessed May 27, 2017].

Tarnopolsky, M.A., 2000. Gender differences in metabolism; nutrition and supplements. *Journal of science and medicine in sport*, 3(3), pp.287–98. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11101268 [Accessed April 15, 2017].

Thien, F.C. et al., 2002. Dietary marine fatty acids (fish oil) for asthma in adults and children. In S. De Luca, ed. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd. Available at: http://doi.wiley.com/10.1002/14651858.CD001283 [Accessed May 27, 2017].

Toft, A.D. et al., 2000. N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. *Journal of applied physiology*, 89(6), pp.2401–2406. Available at: http://jap.physiology.org/content/jap/89/6/2401.full.pdf [Accessed April 11, 2017].

Wang, C. et al., 2006. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *The American journal of clinical nutrition*, 84(1), pp.5–17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16825676 [Accessed March 31, 2017].

Webb, E.C. & Willems, M.E., 2010. Effects of Wearing Graduated Compression Garment during Eccentric Exercise. *Medicina Sportiva*, 14(4), pp.193–198. Available at: http://versita.metapress.com/openurl.asp?genre=article&id=doi:10.2478/v10036-

010-0031-4 [Accessed May 27, 2017].

Wheatley, C.M. et al., 2014. Sex differences in cardiovascular function during submaximal exercise in humans. *SpringerPlus*, 3, p.445. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25191635 [Accessed May 22, 2017].

Yokoyama, M. et al., 2007. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *The Lancet*, 369(9567), pp.1090–1098. Available at: http://www.sciencedirect.com/science/article/pii/S0140673607605273 [Accessed March 31, 2017].

Zebrowska, A. et al., 2015. Omega-3 fatty acids supplementation improves endothelial function and maximal oxygen uptake in endurance-trained athletes. *European journal of sport science*, 15(4), pp.305–314. Available at: http://www.tandfonline.com/action/journalInformation?journalCode=tejs20 [Accessed April 8, 2017].