Effect of omega-3 fatty acid supplementation on inflammation, muscle damage and exercise performance in athletes: A systematic review and meta-analysis

J Viljoen

orcid.org/ 0000-0002-5739-3074

Dissertation submitted in fulfilment of the requirements for the degree *Masters of Science in Nutrition* at the North-West University

Supervisor: Prof L Havemann-Nel

Co-supervisor: Dr C Ricci

Graduation: May 2019

Student number: 23437820

ACKNOWLEDGMENTS

Firstly, I would like to thank my supervisor Prof Lize Havemann-Nel; it has been an honour to be your postgraduate student and I am grateful for all your support throughout my journey. Thank you for your patience and all the valuable lessons you have shared with me, you are a remarkable mentor and your lessons and leadership is what shaped me into the academic I am today.

Furthermore, I would like to express my sincerest gratitude to the following individuals:

To my parents, you are my greatest blessing. Thank you for always believing in me and encouraging me to pursue my dreams, your support and unconditional love from the smallest to the biggest things means everything to me.

To my co-supervisor, Dr Cristian Ricci, thank you for your significant contribution and help in completing this dissertation; for your patience and teaching, your expertise helped so much and it is much appreciated.

I would also like to thank the National Research Foundation (NRF) for the financial support.

Most importantly I would like to thank the God of all creation, seeing His hand in my journey and research is such a privilege and I will always be grateful for every opportunity He has given me throughout the years and the years to come. My passion and inspiration for research originated from His marvellous creation. **For from Him and to Him are all things. In Him we live and move and have our being.**

ABSTRACT

Background: Large volumes of unaccustomed, intense exercise in athletes cause increased muscle damage, inflammation and suppression of the immune system resulting in delayed exercise recovery, overtraining syndrome, and compromised exercise performance. Available evidence suggests that omega-3 (n-3) polyunsaturated fatty acids (PUFAs) may alter the exercise-induced inflammatory response and have immunomodulatory effects in athletes and active individuals, however the evidence regarding this topic is contradicting. Therefore, the aim of this Masters study was to perform a systematic review and meta-analysis (where possible) of the literature regarding the effect of n-3 PUFA supplementation on exercise-induced inflammation, muscle damage and exercise performance in athletes.

Methods: Seven electronic databases were searched and 18 randomised controlled trials were included for analysis. Meta-analytical synthesis was performed using a random effect analysis to calculate the effect size of n-3 PUFA supplementation on markers of inflammation (Tumor Necrosis Factor [TNF]-α, Interleukin 2 [IL-2], -6 [IL-6], -4 [IL-4], -10 [IL-10] and C-reactive protein [CRP]), muscle damage (creatine kinase [CK]) and exercise performance (time trial time and time to exhaustion). A sensitivity analysis was done excluding one study at a time. Heterogeneity was evaluated by the I-square index and Cochrane's Q test.

Results: A suggestive trend for a statistically significant beneficial effect of n-3 PUFA supplementation on anti-inflammatory cytokine IL-10 (SMD = 0.74, 95% CI: -0.08 to 1.56; P = 0.075) was observed. Moreover, this study also observed a suggestive trend for a statistically significant reduction of the pro-inflammatory marker CRP (SMD = -2.03, 95% CI: -4.31 to 0.25, P = 0.081). However, we observed no effect of n-3 PUFA supplementation on inflammatory markers TNF-α, IL-2, IL-4, IL-6, muscle damage marker CK or exercise performance measurements.

Conclusion: Although n-3 PUFA supplementation demonstrated no beneficial effects on exercise performance and some inflammatory markers (TNF-α, IL-2 and IL-4), the potential increase in the anti-inflammatory cytokine IL-10 as well as the reduction of CRP concentrations suggest that n-3 supplementation potentially enhances aspects of the immune system and improve exercise recovery.

Keywords: Omega-3 polyunsaturated fatty acids, inflammation, muscle damage, exercise performance, athletes, active individuals

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ATP	Adenosine triphosphate
bpm	Beats per minute
CAT	Catalase
CHO	Carbohydrates
CK	Creatine kinase
COX	Cyclooxygenase
CRP	C-reactive protein
cSOD	Cytosolic superoxide dismutase
Cu,ZnSOD	Coper-zinc superoxide dismutase
DHA	Docosahexaenoic acid
EIMD	Exercise-induced muscle damage
eNOS	Endothelial nitric oxide synthase
ES	Effect size
EPA	Eicosapentaenoic acid
g	Grams
g/d	Grams per day
GPX	Glutathione peroxidase
GSH	Glutathione
GSSG	Oxidized glutathione
H_2O	Water

H_2O_2	Hydrogen peroxide
HETE	Hydroxyeicosatetraenoic acids
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-10	Interleukin-10
LCPUFA’s	Long chain polyunsaturated fatty acids
LDM	Lipid-derived mediators
LOX	Lipoxygenase
LT	Leukotriene
LX	Lipoxins
MaR	Marsins
Max	Maximum
mcg	Micrograms
mg	Milligrams
Min	Minimum
MnSOD	Manganese superoxide dismutase
n	Number of participants
n-3	Omega 3
n-6	Omega 6
NADPH	β-nicotinamide adenine dinucleotide phosphate
NO	Nitrogen oxide

O_2^-	Superoxide
$OH^\bullet$	Hydroxyl radicals
$ONOO^-$	Peroxynitrite
OTS	Overtraining syndrome
OX	Xanthine oxidase
PD	Protectins
PG	Prostaglandins
PGE_2	Prostaglandins E series
PLA_2	Phospholipase A_2
PM	Plasma membrane
PMNs	Polymorphonuclear leukocytes
ROS	Reactive oxygen species
Rv	Resolvins
SD	Standard Deviation
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor – alpha
VDAC	Voltage-dependent anion channel

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 Background and rationale

Regular physical activity and/or exercise causes numerous physiological adaptations depending on the volume, intensity, and frequency of exercise (Slattery *et al.*, 2015). Active individuals (recreational to elite level) regularly engage in physical activity and exercise to improve amongst other health and/or exercise performance (Hackney & Koltun, 2012). Similarly, to other stressors such as disease, environmental conditions (e.g. cold temperatures) or trauma, exercise, particularly high volumes of intensive exercise act as a stress on the body that disturbs the homeostatic balance (Slattery *et al.*, 2015). The repetitive contraction of the skeletal muscle during physical activity and/or exercise can result in exercise-induced muscle damage (EIMD) (Baumert *et al.*, 2016). EIMD can result from damage to the muscle structure (e.g. muscle fibre tears) due to mechanical stress as well as from metabolic stress which comprises of exercise-induced oxidative stress and inflammation (Brancaccio *et al.*, 2010; Panza *et al.*, 2015). Oxidative stress is characterized by an increased production of reactive oxygen species (ROS). ROS are highly unstable molecules due to an unpaired electron (Pingitore *et al.*, 2015; Steinbacher & Eckl, 2015). The production of moderate levels of ROS in response to moderate exercise influences the antioxidant system positively by stimulating endogenous antioxidant production (Steinbacher & Eckl, 2015). Therefore, the increase in ROS in response to repeated bouts of moderate exercise results in an adaptation in the endogenous antioxidant system within the skeletal muscle (Rowlands *et al.*, 2012; Slattery *et al.*, 2015), enabling the body to neutralise ROS production during exercise (Urso & Clarkson, 2003).

In contrast to regular moderate exercise, prolonged or vigorous exercise often results in excessive ROS production causing oxidative stress (Slattery *et al.*, 2015). Oxidative stress, therefore, is caused when ROS production exceeds the endogenous cellular antioxidant capacity (Pingitore *et al.*, 2015). A significant increase in ROS production, due to intense exercise or muscle injury, results in EIMD as mentioned above. Following EIMD there is an initiation of multiple cellular and molecular processes, including the activation of systemic inflammatory pathways, to restore the structure and function of the skeletal muscle (Fullerton *et al.*, 2014; Hensley *et al.*, 2000; Philippou *et al.*, 2012; Steinbacher & Eckl, 2015). Activation of inflammatory pathways involves amongst other the production of soluble mediators such as C-reactive protein (CRP), pro- and anti-inflammatory eicosanoids (e.g. prostaglandins and resolvins) as well as cytokines (e.g. interleukin and interferons) (Calder, 2015). The process of inflammation eliminates excessive ROS production and promotes tissue repair, therefore enabling the physiological adaptation process (Gilroy & De Maeyer, 2015; Slattery *et al.*, 2015). However, the resolution of inflammation in the

skeletal muscle is crucial for recovery and failure to adequately resolve the process of inflammation can result in delayed onset of muscles soreness (DOMS) (Kanda *et al.*, 2013). Moreover, chronic inflammation results in a condition called overtraining syndrome (OTS) which is characterized by decreased exercise performance and muscular strength, chronic fatigue, increased muscle soreness, a compromised immunity and the inability to train (Hackney & Koltun, 2012). The presence of DOMS and OTS impact recovery, training and exercise performance in competitive athletes and have health implications (i.e. depressed mood, central fatigue and resultant neurohormonal changes) (Hackney & Koltun, 2012; Kanda *et al.*, 2013). Therefore, active individuals and athletes are constantly searching for different nutritional strategies, including the intake of nutrients and supplementation to enhance recovery and improve exercise performance (Da Boit *et al.*, 2017).

The consumption of n-3 PUFA have been implicated in the modulation of immune and exercise-induced inflammatory responses (Andrade *et al.*, 2007). Dietary intake of n-3 fatty acids has been shown to alter the phospholipid membrane composition in immune cells and muscle cells (Calder, 2006). The increased consumption of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derived from n-3 PUFAs does result in a decreased synthesis of pro-inflammatory mediators, which in turn, increase the synthesis of anti-inflammatory factors derived from EPA and DHA (Calder, 2017).

In fact, there is a rising body of literature reporting on the different effects of n-3 PUFA supplementation on inflammation, muscle damage and exercise performance in active individuals, but sometimes with conflicting results (Andrade *et al.*, 2007; Atashak' *et al.*, 2013; Bloomer *et al.*, 2009; Da Boit *et al.*, 2015; Delfan *et al.*, 2015; Gray *et al.*, 2014; Gray *et al.*, 2012; Nieman *et al.*, 2009; Oostenbrug *et al.*, 1997; Radoman *et al.*, 2015; Ránky *et al.*, 2017; Saiiari & Boyerahmadi, 2014; Santos *et al.*, 2013). Omega-3 PUFA supplementation for 2-6 weeks has shown to attenuate levels of the pro-inflammatory marker TNF-α in elite male paddlers (Delfan *et al.*, 2015), male endurance athletes (Saiiari & Boyerahmadi, 2014) and exercise-trained men (Bloomer *et al.*, 2009). However, Santos *et al.* (2013); Skarpańska-Stejnborn *et al.* (2010); Toft *et al.* (2000) showed no effect of n-3 PUFA supplementation on TNF-α in marathon athletes, male rowers and endurance trained males, respectively. Omega-3 supplementation for six weeks has further shown to increase pro-inflammatory marker IL-2 in competitive male swimmers, recreational athletes and male and female swimmers (Andrade *et al.*, 2007; Da Boit *et al.*, 2015; Gray *et al.*, 2012) but fail to show any effect on endurance-trained males (Santos *et al.*, 2013; Toft *et al.*, 2000). Moreover, a decrease in pro-inflammatory marker CRP was observed after supplementation with EPA and DHA ranging from 2.5 – 3 g/d in exercise-trained males, bodybuilders and military personnel, respectively (Bloomer *et al.*, 2009; Hosseini *et al.*, 2015;

Lembke *et al.*, 2014; Santos *et al.*, 2012) whereas no effect was observed in handball players and trained cyclists, respectively (Atashak *et al.*, 2012; Nieman *et al.*, 2009).

With regards to the effect of n-3 omega supplementation on muscle damage, Hosseini *et al.* (2014) observed decreased levels of muscle damage marker Creatine Kinase (CK) following omega-3 supplementation in bodybuilder athletes. However, in contrast an increase in CK was observed in military personnel and recreational active individuals after the supplementation of n-3 PUFAs (Gray *et al.*, 2014; Santos *et al.*, 2012).

Cytokine IL-6 is known to have pro-inflammatory effects as it is one of the most potent mediators in the acute phase response but is also known to be an anti-inflammatory mediator due to its properties to restrict cytokine production (Moldoveanu *et al.*, 2001). Capó *et al.* (2014); Delfan *et al.* (2015) observed an increase in IL-6 after 4-8 weeks of n-3 PUFA supplementation in football and endurance rowing athletes, respectively. Additionally, no change in IL-6 was observed after administration of supplementation for 6-8 weeks in male cyclists, soccer players and endurance runners, respectively (Nieman *et al.*, 2009; Radoman *et al.*, 2015; Toft *et al.*, 2000).

Studies reporting on the effect of n-3 PUFA supplementation on anti-inflammatory cytokine IL-10 have not come to a clear conclusion (Capó *et al.*, 2014; Da Boit *et al.*, 2015; Delfan *et al.*, 2015; Santos *et al.*, 2013). An increase in IL-10 was observed in elite paddlers after 3.6 g/d n-3 PUFA supplementation for 4 weeks (Delfan *et al.*, 2015). In contrast, Santos *et al.* (2013) found a decrease in IL-10 after supplementation of 3 g/d in marathon runners. Whereas, Capó *et al.* (2014); Da Boit *et al.* (2015) observed no effect in football players and exercise-trained individuals, respectively.

Finally, the effect of n-3 PUFA supplementation on exercise performance has also been explored. Lewis *et al.* (2015) observed an increase in time trial performance following 3 weeks of fish oil supplementation compared to a placebo group, with the remainder of studies showing no difference for n-3 supplementation on time trial performance (Da Boit *et al.*, 2015; Hingley *et al.*, 2017; Oostenbrug *et al.*, 1997) or time to exhaustion (Buckley *et al.*, 2009; Huffman *et al.*, 2004).

As demonstrated above, evidence regarding the outcomes of n-3 PUFA supplementation on inflammation, muscle damage, and exercise performance is inconclusive. This could be attributed to the inclusion of different population groups, variability in the duration and dosage of supplementation as well as differences in study designs, which resulted in unequal sample sizes and methodological heterogeneity. A systematic review of the current literature on the effect of n-3 PUFA supplementation on inflammation, muscle damage and exercise performance may be warranted.

1.2 Research aims and objectives

Therefore, the aim of this Masters study was to perform a systematic review and meta-analysis (where possible) of the literature regarding the effect of n-3 PUFA supplementation on inflammation, muscle damage and exercise performance.

The specific objectives were to systematically review the effect of n-3 PUFA supplementation compared to a placebo/control on:

1. The markers of inflammation including cytokines (TNF-α, IL-2, -4, -6 and 10) and acute-phase proteins (CRP) in recreational and competitive athletes.

2. Muscle damage marker (creatine kinase) in recreational and competitive athletes and;

3. Exercise performance measured through time trials and time to exhaustion in recreational and competitive athletes.

1.3 Research team

The following table is a summary of the research team, their expertise, and their role.

Table 1-1: Team members, their roles and expertise

Name	**Role in the study**
Johnine Viljoen	MSc student responsible for writing the protocol, study selection, data extraction, statistical analysis and data storage, quality assessment and writing of the final manuscript.
Prof Lize Havemann-Nel	Supervisor of MSc student with expertise on the research topic. Responsible for supervising all the student activities.
Dr Cristian Ricci	Co-supervisor of MSc student with expertise in writing systematic reviews and conducting meta-analyses. Co-responsible for study selection, data extraction, statistical analysis and data storage, and quality assessment.

1.4 Structure of dissertation

This dissertation is presented in article format and consists of four chapters. Chapter one provides a rationale for the study, outlines the aim and objectives and gives an overview of the research team. Chapter two presents the literature review that provides an overview of exercise-induced muscle damage and inflammation, including the mechanisms involved in exercise-induced muscle damage. The role of n-3 PUFA in inflammation, and a summary of the existing literature on the effect of n-3 PUFA supplementation on markers of inflammation (i.e. cytokines and acute phase proteins), muscle damage (creatine kinase) and exercise performance is also included. Chapter three of the dissertation consists of the research manuscript entitled: “Effect of Omega-3 fatty acid supplementation on inflammation, muscle damage and exercise performance in active individuals: A systematic review and meta-analysis”. The manuscript is written according to the specification of the International Journal of Sports Nutrition and Exercise Metabolism (IJSNEM). In the final chapter (Chapter four) the researcher provides a short summary and conclusion, acknowledges the limitations and makes recommendations based on findings. The references of chapter one, two and four are according to the North-West University Harvard style and are listed in the reference list, following chapter four.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Regular exercise and physical activity are commonly associated with a healthy lifestyle as well as increased exercise performance depending on the volume, intensity and frequency of each exercise bout (Petersen & Pedersen, 2005; Slattery *et al.*, 2015). There is a well-known association between intense, prolonged and repeated eccentric contractions and exercise-induced muscle damage (EIMD) and inflammation (Kendall & Eston, 2002). EIDM can occur in both recreational and competitive athletes. With competitive athletes, muscle damage are often related to a sudden increase in the volume or intensity of training regime or following prolonged injury and inadequate rest (Kendall & Eston, 2002). For recreational athletes, a single bout of exercise involving strenuous, unaccustomed eccentric muscle contraction may produce significant muscle soreness and damage (Kendall & Eston, 2002). Following exercise-induced skeletal muscle damage, multiple cellular and molecular processes are activated to restore the structure and function of skeletal muscle (Philippou *et al.*, 2012). These processes typically involve an inflammatory response at the local site of damage within the muscle and systemic within the body before the resolution and completion of muscle repair or regeneration (Philippou *et al.*, 2012). The overall outcome of the inflammatory process can either be detrimental with the induction of prolonged inflammation and further muscle damage leading to conditions such as overtraining syndrome (OTS) and delayed onset of muscle soreness (DOMS) or beneficial through the active termination and the promotion of muscle repair and regeneration (Calder, 2015; Hackney & Koltun, 2012; Kanda *et al.*, 2013; Philippou *et al.*, 2012). Thus, the interaction between systemic and muscle-derived cytokines acting as positive and/or negative regulators coordinating the local and systematic inflammatory-related events and modulate the muscle repair process determine whether the inflammatory response will be detrimental or beneficial (Philippou *et al.*, 2012). To attenuate an excessive inflammatory reaction and promote the regenerative process the crucial balance between pro- and anti-inflammatory cytokines should be maintained (Peake *et al.*, 2005; Philippou *et al.*, 2012).

Recently omega-3 (n-3) polyunsaturated fatty acid (PUFA) has been suggested to play a key immunomodulatory role in the inflammatory response (Calder, 2015; Serhan *et al.*, 2015a). n-3 PUFA supplementation is one of the nutrition strategies recently being used within the sports nutrition community for its possible effect on inflammation, muscle damage and exercise performance but the evidence regarding n-3 PUFA supplementations effect is inconclusive (Atashak' *et al.*, 2013; Buckley *et al.*, 2009; Da Boit *et al.*, 2015; Gray *et al.*, 2014; Gray *et al.*, 2012; Hingley *et al.*, 2017; Hosseini *et al.*, 2015; Jakeman *et al.*, 2017). Therefore, insight on

exercise-induced muscle damage and inflammation, n-3 PUFAs role during inflammation and the effect of n-3 supplementation will be explored in the following sections.

2.2 Exercise-induced oxidative stress

Regular exercise has been known to improve muscle strength and/or resistance to muscle fatigue (Paulsen *et al.*, 2012). This is due to the adaptive nature of the skeletal muscle to stressors that disturbs the homeostatic balance within the muscles and the body (Slattery *et al.*, 2015; Tidball, 2011). Physiological adaptations help retain homeostasis through the upregulation of biological systems (i.e. endogenous antioxidant system and inflammatory response) to help aid the recovery process (Slattery *et al.*, 2015). Maintaining a balance between exercise-induced stress and recovery is a crucial part of physiological adaptation and an imbalance can leave the body in a maladaptive state (Slattery *et al.*, 2015). When the body is left in a maladaptive state due to inadequate recovery the skeletal muscles are more prone to fatigue and temporary weakening (Paulsen *et al.*, 2012). The repetitive contraction of the skeletal muscle during exercise causes mechanical and metabolic disturbances, thus resulting in EIMD (Peake *et al.*, 2005; Slattery *et al.*, 2015; Tee *et al.*, 2007). EIMD is a common occurrence following a single bout of eccentric and/or intense prolonged training and result in initial damage due to mechanical stress (i.e. muscle tears) of muscle contraction and more systemic related changes in later events due to metabolic stress (i.e. oxidative stress and inflammation) (Brancaccio *et al.*, 2010; Panza *et al.*, 2015). Oxidative stress is the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which exceeds the capacity of the endogenous antioxidant defence system (Yavari *et al.*, 2015). It is known that oxidative stress and the associated signalling responses are closely related to inflammation and inflammatory signalling (Fisher-Wellman & Bloomer, 2009; Slattery *et al.*, 2015). Inflammation is the body's natural defence mechanism to infection, pathogens and other insults such as exercise-induced injury and oxidative stress (Calder, 2008; Slattery *et al.*, 2015). Intense or unaccustomed exercise can cause oxidative stress through the overproduction of ROS and RNS, resulting in inflammation (Markworth *et al.*, 2013; Slattery *et al.*, 2015). The activation of the inflammatory response involves the release of inflammatory mediators from muscle and immune cells at the site of injury (Serhan *et al.*, 2015b). These soluble mediators can be pro- or anti-inflammatory and play a key role in the initiation and resolution of inflammation (Serhan & Petasis, 2011). In contrast to intense or unaccustomed exercise, regular moderate exercise has beneficial effects through adaptation by the up-regulation of the endogenous antioxidant response (Pingitore *et al.*, 2015). Therefore, the homeostatic balance between ROS production and the antioxidant system is necessary for maintaining healthy adaptation to exercise (Slattery *et al.*, 2015).

2.2.1 Reactive oxygen species and oxidative stress

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), also known as free radicals, are highly unstable and reactive molecules due to an unpaired electron (Steinbacher & Eckl, 2015). The most common ROS and RNS generated in skeletal muscle are superoxide (O_2^-), nitrogen oxide (NO), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^\bullet$). These molecules act as biological messengers and signalling molecules (Jackson, 2015; Slattery *et al.*, 2015). The participation of ROS in redox reactions normally results in the oxidation of the molecule (Slattery *et al.*, 2015), which is essential to bioactive signalling (Slattery *et al.*, 2015). When these essential bioactive signalling molecules exceed the balance with reducing agents, these excessed molecules could have detrimental effects on cell structures (Ji *et al.*, 2016). As signalling molecules, ROS and RNS initiate intracellular cascades to promote adaptive responses (Hensley *et al.*, 2000; Slattery *et al.*, 2015). These adaptive responses can be caused by the ROS production during regular moderate exercise where the adaptation in the skeletal muscle and the endogenous antioxidant system takes place (Fisher-Wellman & Bloomer, 2009; Slattery *et al.*, 2015).

At rest and during muscle contraction, O_2^- and NO are the primary free radicals generated by skeletal muscle (Jackson *et al.*, 2007). During contraction of the skeletal muscle, the generation of O_2^- and NO increases **(Figure 2-1)** (Jackson, 2015). The first line of defence against O_2^- radicals is the superoxide dismutase (SOD) enzymes (Powers & Jackson, 2008).

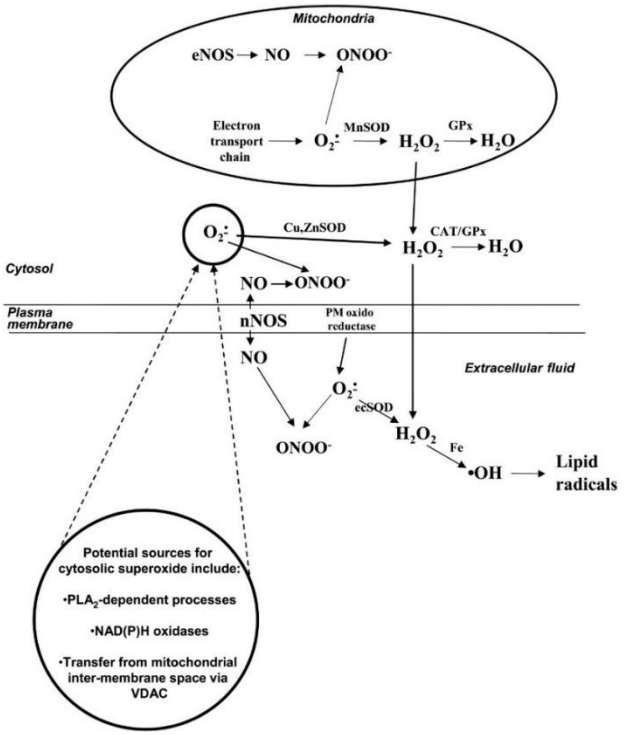


Figure 2-1: Production of reactive oxygen species (ROS) and free radical signalling in the mitochondria and cytoplasm.

eNOS= endothelial nitric oxide synthase; NO= nitrogen oxide; ONOO-= peroxynitrite; O°2 = superoxide; MnSOD = manganese superoxide dismutase; H2O2 = hydrogen peroxide; GPx = glutathione peroxidase; H2O = water; CAT = catalase; Cu,ZnSOD = coper-zinc superoxide dismutase; cSOD = cytosolic superoxide dismutase; Fe = iron; PM = plasma membrane PLA2 = phospholipase A2; NAD(P)H = nicotinamide adenine dinucleotide phosphate; VDAC = voltage-dependent anione channel **(Adapted from Bresciani et al. (2015)).**

There are three SOD enzyme isoforms namely SOD1 also known as copper-zinc superoxide dismutase (Cu,ZnSOD), secondly SOD2, also known as manganese superoxide dismutase (MnSOD) and thirdly SOD3, Cu,ZnSOD (Powers & Jackson, 2008). SOD2 is located within the mitochondria and SOD3 is located in the extracellular space (Powers & Jackson, 2008). Within the mitochondria, O_2^- is converted to H_2O_2 and oxygen (O_2) through the MnSOD enzyme (Collins *et al.*, 2012; Powers & Jackson, 2008). H_2O_2 are further neutralized to H_2O with glutathione (GSH) as a substrate for the glutathione peroxidase (GPx) enzyme (Bresciani *et al.*, 2015). H_2O_2 is known to be the most reactive and reacts with metal ions to generate additional ROS such as $OH^•$ (Hensley *et al.*, 2000; Niess & Simon, 2007). Through the Fenton reaction with iron (Fe^+), H_2O_2 is reduced to $OH^•$ in the extracellular milieu (Bresciani *et al.*, 2015; Powers & Jackson, 2008). $OH^•$ reacts with almost any component in the cell including lipids in the phospholipid membrane (Hensley *et al.*, 2000). The net result of the ROS, $OH^•$, is damaging to cells thus resulting in

oxidative stress and the initiation of the inflammatory process (Hensley *et al.*, 2000; Slattery *et al.*, 2015). Within the membrane interspace Cu,ZnSOD converts O_2^- to form H_2O_2 while in the extracellular milieu the same reaction takes place through the extracellular SOD (Bresciani *et al.*, 2015). In the cytosol, the neutralization of H_2O_2 takes place through the GPx and catalase (CAT) enzymes (Bresciani *et al.*, 2015). Peroxynitrite ($ONOO^-$) is generated through the reaction of NO with O_2-, this reaction is known to be more efficient in scavenging O_2^- than the SOD enzymes (Jackson *et al.*, 2007).

2.2.2 Reactive oxygen species and exercise

The generation of ROS and RNS is mainly mitochondrial driven but can also occur in the cytosol and the extracellular space of the cell (Powers & Jackson, 2008). However, the main source for the formation of ROS and RNS during exercise is the mitochondria through mitochondrial respiration (Hensley *et al.*, 2000; Slattery *et al.*, 2015). During exercise and muscle contraction, oxygen consumption increases in the skeletal muscle (Yavari *et al.*, 2015) resulting in an increase ROS and RNS production (Niess & Simon, 2007; Yavari *et al.*, 2015). Skeletal muscle is a highly specialized tissue which response to external stimuli such as exercise (Steinbacher & Eckl, 2015). Exercise is associated with the increased production of ROS, therefore causing alterations in the redox balance (Slattery *et al.*, 2015). The redox balance is known as the reduction/oxidation potential within a cell and is very well regulated in the body (Fisher-Wellman & Bloomer, 2009). Antioxidant enzymes, SOD, CAT and GPx activity increases during exercise generation of ROS and RNS (Steinbacher & Eckl, 2015). Therefore the antioxidant enzymes increase in response to exercise and are the main defence against ROS and RNS generated during exercise (Niess & Simon, 2007; Slattery *et al.*, 2015; Steinbacher & Eckl, 2015).

Regular moderate exercise has shown to up-regulate antioxidant enzyme activity, therefore, enhancing antioxidant capacity making the well-trained individuals and/or athletes less susceptible to damaging redox reactions than untrained individuals (Slattery *et al.*, 2015). The production of ROS in response to regular moderate exercise influence the antioxidant system positively by increasing antioxidant expression through cellular processes, as shown in **Figure 2-2** (Steinbacher & Eckl, 2015). Therefore, the increase in ROS in response to regular moderate exercise results in an adaptation in the skeletal muscle that involves the up-regulation of the antioxidant enzymes mentioned earlier (Steinbacher & Eckl, 2015), thus neutralizing the ROS (Urso & Clarkson, 2003).

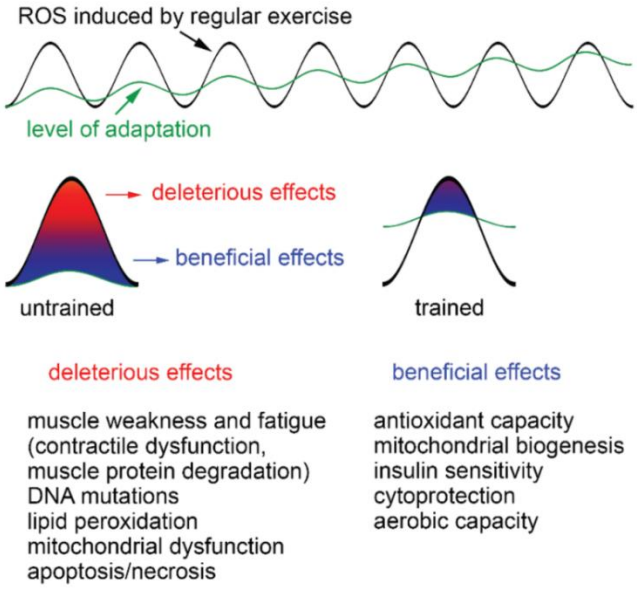


Figure 2-2: Exercise-induced ROS to increase deleterious and beneficial effects.

ROS produced during exercise can have deleterious and beneficial effects depending on the concentration of ROS, duration of ROS exposure and training status if the individual **(Adapted from Steinbacher and Eckl (2015)).**

In contrast to moderate intensity exercise, intense unaccustomed exercise can influence the antioxidant capacity to buffer oxidant production (Slattery *et al.*, 2015). Oxidative stress can result from insufficient antioxidant protection leading to the inability to adapt to the physical activity stimuli (Slattery *et al.*, 2015). During intense unaccustomed exercise, there is an increase in ROS production overwhelming the antioxidant system resulting in a failed attempt to neutralize ROS (Rowlands *et al.*, 2012). Therefore, ROS are produced in greater amounts than the antioxidant system can handle (Rowlands *et al.*, 2012). Resulting in ROS attacking other cellular components such as proteins, DNA and membrane lipids called long-chain polyunsaturated fatty acids (LCPUFA's) **(Figure 2-2)** (Urso & Clarkson, 2003). The attack on LCPUFA's in the cell membrane initiates a chain reaction called lipid peroxidation (Urso & Clarkson, 2003). Lipid peroxidation is the process of oxidative breakdown of LCPUFA's resulting in a change in membrane permeability (Niess & Simon, 2007). Changes in compositions in the membrane can modify membrane fluidity and the pattern of inflammatory mediator production (Calder, 2010). Although the local changes in oxidation-reduction (redox) homeostasis and inflammatory events can be detrimental when not regulated it is part of the process of muscle repair and regeneration (Panza *et al.*, 2015).

2.3 Exercise-induced inflammation

Skeletal muscle is a highly specialized tissue with excellent plasticity in response to mechanical and metabolic stress from exercise (Slattery *et al.*, 2015; Steinbacher & Eckl, 2015). Mechanical stress induced by eccentric exercise that exerts the muscle's ability to maintain homeostasis results in EIMD due to damage to the structural integrity of myofibers and temporary reductions in contractile function (Slattery *et al.*, 2015). Whereas, metabolic stress from prolonged endurance exercise results in the depletion of adenosine triphosphate (ATP) and leakage of extracellular calcium ions (Ca^{2+}) into intracellular space which is responsible for the activation of multiple cellular and molecular processes (Moldoveanu *et al.*, 2001; Philippou *et al.*, 2012). The local response to intense prolonged and/or eccentric exercise is typically characterised by an inflammatory process as well as the beneficial outcome of muscle repair and regeneration (Philippou *et al.*, 2012). The local inflammatory response is dominated by phagocytic cells including neutrophils, macrophages, monocytes and lymphocytes contributing to the clearance of necrotic tissue and cellular debris to repair the muscle (Buckley *et al.*, 2014; Philippou *et al.*, 2012). These inflammatory cells also secrete soluble molecules, mainly cytokines, at the damaged site which coordinate inflammatory-related events and play an active role as positive and/or negative regulators of the muscle inflammatory and repair process (Philippou *et al.*, 2012). Accompanying the local inflammatory response is the systemic response known as acute phase response where acute phase proteins and cytokines also play an important role since, they are not only secreted locally at the site of damage but also systemically in the circulation (Philippou *et al.*, 2012). Cytokines can be pro- and anti-inflammatory and contribute to specific aspects of inflammation based on their predominant action (Philippou *et al.*, 2012; Tidball, 2005). The overall resolution of EIMD and the inflammatory response can either be harmful causing prolonged inflammation and further damage or positive through the active termination of the inflammatory response aiding the repair and regenerative process (Buckley *et al.*, 2014; Philippou *et al.*, 2012). Although the potential effect of cytokines and other cellular and molecular events involved in exercise-induced inflammation has been well research, the direct mechanism of the resolution, repair and regeneration process with regards to skeletal muscle is still not well understood (Moldoveanu *et al.*, 2001; Philippou *et al.*, 2012; Steinbacher & Eckl, 2015; Tidball, 2005).

2.3.1 Exercise-induced muscle damage (EIMD)

Skeletal muscle damage can be caused by numerous events which can either be internal such as ischemia and metabolic deficits or through external events such as mechanical overloading and stretching of the muscle through exercise models or a combination of the two as occurs in prolonged and/ or eccentric exercise (Philippou *et al.*, 2012). Eccentric resistance training has been used to explore contraction-induced muscle damage and the cellular and molecular response to the damage (Hyldahl *et al.*, 2017). Eccentric muscle contraction during resistance training leads to functional and structural disruptions due to mechanical disturbances within the contractile system of the muscle fibre (Baird *et al.*, 2012). These mechanical disturbances are characterized by the disruptions of myofilament structures in sarcomeres and damage to the sarcolemma, loss of fibre integrity and leakage of muscle proteins and enzymes such as creatine kinase (CK) into blood serum (Baird *et al.*, 2012; Paulsen *et al.*, 2012). Howatson and Van Someren (2008) proposed the following mechanical damage hypothesis cause by mechanical loading on the myofibers. The skeletal muscles fibres consist of myofibrils composed of actin and myosin filaments, repeated units are known as sarcomeres as shown in **Figure 2-3** (Pillon *et al.*, 2013). During eccentric contractions, the sarcomeres lengthen in a non-uniform way resulting in myofilaments being stretched preventing sarcomere overlapping (Howatson & Van Someren, 2008; Hyldahl & Hubal, 2014). Consequently, the stretching of sarcomeres beyond the point of overlapping results in Z-band streaming also known as a term “popping” causing failure of structures reducing the ability of the muscle to generate force **(Figure 2-3)** (Howatson & Van Someren, 2008; Hyldahl & Hubal, 2014). Moreover, the above-mentioned increases membrane permeability leading to excitation contraction (E-C) coupling dysfunction (Hyldahl & Hubal, 2014). Following the initial damage to myofibrillar and/or to E-C coupling elements, metabolic muscle disturbances is thought to result in adenosine triphosphate (ATP) depletion resulting in uncontrolled CA^{2+} release from sarcoplasmic reticulum and an increase in efflux of cytosolic proteins and enzymes including CK (Baird *et al.*, 2012; Paulsen *et al.*, 2012; Proske & Morgan, 2001).

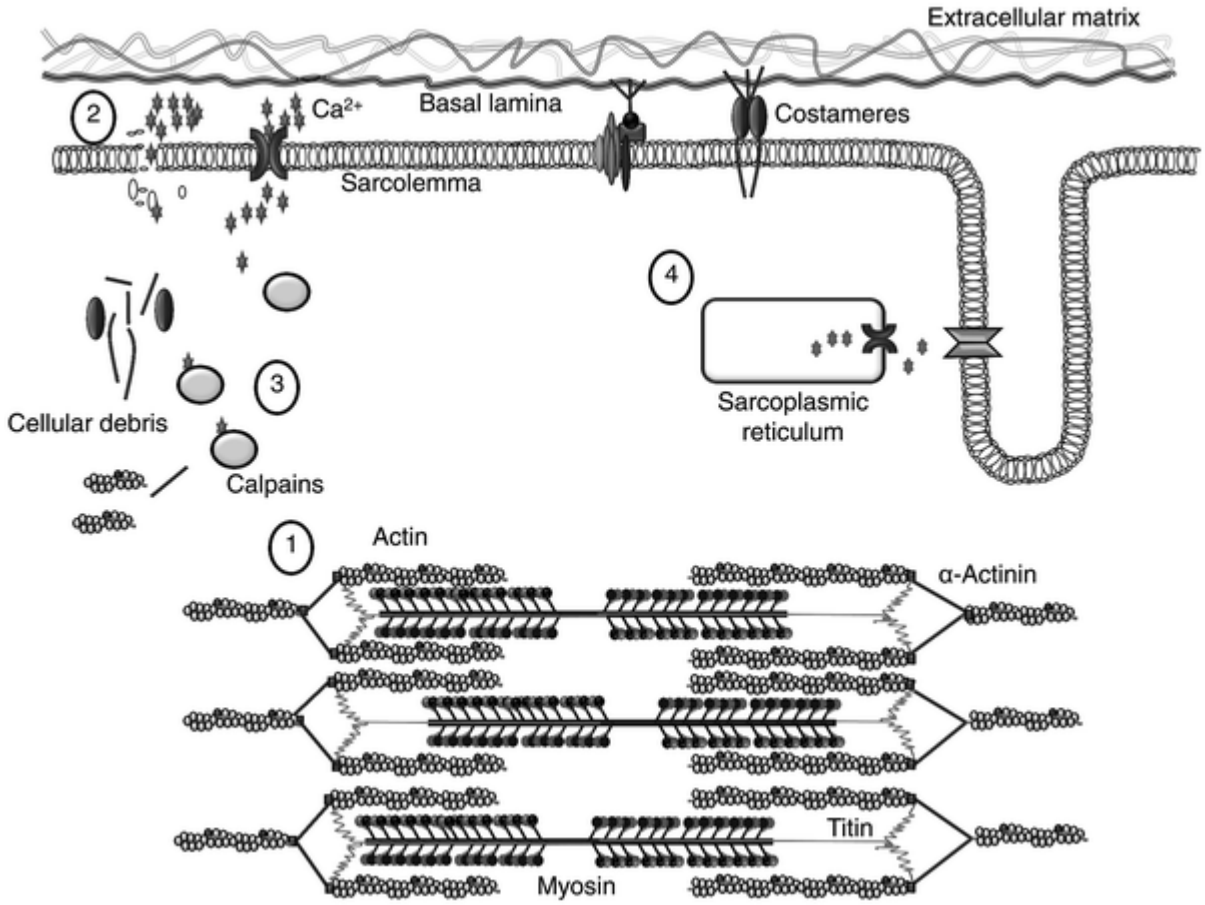


Figure 2-3: Initial phase of EIMD due to eccentric muscle contraction

During eccentric muscle contraction sarcomere overlapping takes place in a non-uniform way preventing filament overlapping leading to sarcomere "popping". (1) Z-line streaming due to sarcomere stretching beyond optimum overlap of actin and myosin filaments, (2) this is followed by an increase membrane permeability of sarcolemma and E-C coupling disfunction, (3) different Ca^{2+}-sensitive proteases (calpains) are activated due to extracellular Ca^{2+} influx into the muscle fibre, (4) E-C coupling dysfunction leading to muscle damage. **(Adapted from Baumert *et al.* (2016))**

In contrast to eccentric exercise, intense prolonged exercise such as endurance exercise does not elicit mechanical muscle damage per se (Baird *et al.*, 2012). Rather, the EIMD is due to metabolic deficiencies within the contracting muscle (Tee *et al.*, 2007). During exhaustive exercise such as endurance exercise, there is an increase in metabolic flux through the glycolytic and oxidative metabolic pathways to match ATP synthesis to the rate of ATP hydrolysis (Tee *et al.*, 2007). The above mention could possibly lead to ATP depletion resulting in the leakage of extracellular calcium ions into intracellular space, due to both Ca^{2+}-ATPase and Na-K-ATPase pump dysfunction (Baird *et al.*, 2012; Tee *et al.*, 2007). Thus, leading to leakage of cytosolic proteins and enzymes including CK (Baird *et al.*, 2012).

Creatine kinase is a compact enzyme found in the cytosol and mitochondria of high energy demand tissues (e.g. skeletal muscles) that forms the core of the phosphocreatine (PCr) circuit (Baird *et al.*, 2012). Moreover, CK is important for the regeneration of cellular ATP through catalysing the reversible phosphorylation of creatine to phosphocreatine and of ADP to ATP (Baird *et al.*, 2012). When intracellular ATP levels are depleted, the release of CK is initiated to aid energy demand, this process is critical for the maintenance of energy supply, increasing the enzymes levels in the blood (Baird *et al.*, 2012). Along with the increased production of CK, there is a release of ROS from the contracting muscles activating the secondary phase of muscle damage (Powers & Jackson, 2008).

The secondary phase of muscle damage due to the metabolic disturbances are characterized by the secretion of immune cells and the activation of the inflammatory response (Hyldahl et al., 2017; Hyldahl & Hubal, 2014). The inflammatory response is known to be a self-regulating process by activating negative feedback mechanisms such as the production of anti-inflammatory mediators following the inhibition of pro-inflammatory mediators (Calder, 2010; Serhan *et al.*, 2015a). The balance between the pro- and anti-inflammatory mediators released by immune and muscle cells are crucial for regulation and regeneration of inflammation (Philippou *et al.*, 2012). After the inflammatory cascade is activated, there is an up-regulation of the pro-inflammatory chemical messengers including cytokines, lipid-derived mediators and chemokines to promote the resolution and repair of the skeletal muscle (Buckley *et al.*, 2014; Slattery *et al.*, 2015). Thus leading to the mobilization and infiltration of phagocytic cells (neutrophils and macrophage/monocytes) which in return secrete proteolytic enzymes and ROS to remove necrotic tissue and cellular debris (Forbes & Rosenthal, 2014; Slattery *et al.*, 2015). The increase in circulating leukocytes is dependent on the intensity, duration and volume of exercise session (Carbal-Santos *et al.*, 2015; Gleeson, 2007). Prolonged, intensive and/or eccentric exercise as mentioned earlier causes exercise-induced skeletal muscle injury resulting in an inflammatory response followed by the resolution via anti-inflammatory cytokines and specialized pro-resolution

mediators (SPMs) (Markworth *et al.*, 2013; Serhan & Recchiuti, 2012). In broad terms, resolution can be defined as the rate of polymorphonuclear leukocytes (PMNs) clearance until their absent at the site of injury (Buckley *et al.*, 2014). Moreover, resolution of inflammation is recently known as an active process (Ariel & Serhan, 2007; Dalli *et al.*, 2013; Serhan *et al.*, 2015b) driven by the anti-inflammatory cytokines and lipid-derived mediators to restore homeostasis by regulating cellular events to clear inflammation rapidly (Gilroy & De Maeyer, 2015).

There are two main steps in the acute inflammatory response, initiation and termination (Buckley *et al.*, 2014; Serhan & Recchiuti, 2012). There are a few key steps from the initiation to the termination of inflammation to be considered and these include 1) removal of the initiating stimuli; 2) the breakdown of the survival signals and inhibition of the pro-inflammatory signalling pathways; 3) apoptosis of PMNs; 4) activation of efferocytosis by tissue and monocyte-derived macrophages, and 5) the termination of the inflammatory response via the resolution (Buckley *et al.*, 2014). The initiation process of the inflammatory response to muscle injury is characterized by the immediate influx of PMNs followed by the termination via phagocytosis and efferocytosis via monocytes-macrophages (Buckley *et al.*, 2014; Koch, 2010; Ortega, 2008). Phagocytosis is the process of PMNs to bind, engulf and destroy pathogens (Koch, 2010) whereas efferocytosis is the removal of apoptotic and necrotic cells (Serhan & Recchiuti, 2012).

2.3.2 The cytokine response to exercise

There are multiple mediators involved in the initiation a termination phase of the inflammatory response including pro- and anti-inflammatory cytokines **(Table 2-1)** (Petersen & Pedersen, 2005). Cytokines are a group of proteins known to mediate the inflammatory response to pathological stimuli such as exercise-induced tissue damage (Peake *et al.*, 2005). The initiation phase stimulated by the EIMD as a local response consists of the release of the production of lipid-derived mediators known as leukotrienes (LTB_4) and prostaglandins (PGE_2 and PGI_2) derived from arachidonic acid (AA) imbedded in the phospholipid membranes leading to PMN recruitment (Calder, 2017; Silva & Macedo, 2011). Along with the production of the above-mentioned lipid-derived mediators, the release of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) are activated as well as the ROS, nitric oxide (Buckley *et al.*, 2014). These pro-inflammatory cytokines also activate the systematic response known as the acute phase response (APR) where C-reactive protein (CRP) is produced from hepatocyte in the liver (Gruys *et al.*, 2005; Kasapis & Thompson, 2005). The cytokine IL-6 which is known to have pro-inflammatory properties due to its initiation of the APR and production of CRP and interleukin-2 (IL-2) as well as anti-inflammatory properties by the initiation of anti-inflammatory cytokines interleukin-4 (IL-4) and interleukin-10 (IL-10) (Philippou *et al.*, 2012). After the pro-inflammatory mediators are released the production of IL-6 increases

activating the production of anti-inflammatory mediators, IL-4 and -10, respectively, into the circulation (Calder, 2017; Philippou *et al.*, 2012). These cytokines IL-4 and IL-10 in return drive to attenuate the inflammatory response and promote muscle repair and regeneration (Philippou *et al.*, 2012; Pillon *et al.*, 2013). Moreover, IL-10 exerts its anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines TNF-α and IL-1β (Philippou *et al.*, 2012). Therefore, promoting the resolution and repair of the damage skeletal muscle. Failure to resolve the inflammatory response to EIMD can cause symptoms such as DOMS or if overtraining and inadequate recovery continues resulting in a chronic inflammatory state known as overtraining syndrome (OTS), respectively (Hackney & Koltun, 2012; Kim & Lee, 2014).

2.3.3 Delayed onset of muscle soreness and overtraining syndrome

Delayed onset of muscle soreness (DOMS) is one of the well-known symptoms of EIMD (Howatson & Van Someren, 2008). The symptoms caused by DOMS includes strength loss, pain (i.e. peak 24-48 hours after exercise and subsides within 96 hours), muscle tenderness, stiffness and swelling, these symptoms can continue for 3-7 days depending on the intensity and volume of the exercise (Kim & Lee, 2014). The exact cause of DOMS is still unclear but it has been proposed that several combined factors such as connective tissue damage surrounding the muscle, muscle temperature, inflammatory response, ROS, and NO causes DOMS (Kim & Lee, 2014). As already explained above the physical mechanical damage (e.g. muscle tears) to the muscle leads to an acute inflammatory response (Howatson & Van Someren, 2008). The production of pro-inflammatory cytokines, prostaglandins, leukotrienes and ROS activates inflammatory cells such as neutrophils and monocytes (Kim & Lee, 2014). In response to the accumulation of the inflammatory cells at the site of damaged muscle there is a further increase in of prostaglandins and leukotrienes along with bradykinin which are potent inflammatory mediators, respectively (Kim & Lee, 2014). This increase in the inflammatory mediator bradykinin activates phospholipase A_2 stimulating the production of arachidonic acid (AA), thus increasing the production prostaglandins and leukotrienes (Brentano & Martins Kruel, 2011; Kim & Lee, 2014). The roles of prostaglandins, leukotrienes and bradykinin in DOMS include the direct interaction with type III and IV afferent nerve fibres through nociceptor (i.e. pain receptors), increased vascular permeability resulting in adhesion of neutrophils in the damaged site and the increase in production of ROS causing further damage (Kim & Lee, 2014). By the time the above mention is activated, muscle swelling resulting in increased intramuscular pressure and sensitivity of type III and IV fibres already occurred therefore muscle soreness is perceived (Brentano & Martins Kruel, 2011; Kim & Lee, 2014). It is important to recognize that DOMS has an important role in the adaptation to exercise-induced stress and inflammation, but failure of adequate recovery and an increase in intense prolonged and/or eccentric exercise can leave the muscle

and body in chronic inflammatory state ultimately leading overtraining syndrome (OTS) (Brentano & Martins Kruel, 2011; Hackney & Koltun, 2012).

Overtraining syndrome can be defined as a mal-adaptive state in athletes due to excessive training loads along with an increased volume and intensity exceeding the individual's ability to recover (Hackney & Koltun, 2012). The mal-adaptive state of the athlete is not just physiological but also behavioural and/or emotional conditions resulting in a persistent decline in physical performance capacity (Hackney & Koltun, 2012; Meeusen *et al.*, 2013). The prevalence of OTS among athletes ranges from approximately 10 – 37% depending on the intensity and type of exercise as well as the age of the athlete (Meeusen *et al.*, 2013). Although OTS affects the body in many ways, this section will only concentrate on the effect of OTS on the inflammation related to the immune system. Once more the exact physiological mechanism responsible for inducing OTS is unknown but the cytokine hypothesis by Dr Lucille Lakier Smith will be discussed. Due to excessive exercise training known as overtraining there is a high level of musculoskeletal loading from exercise resulting in EIMD (Hackney & Koltun, 2012; Hyldahl & Hubal, 2014). As mention earlier EIMD results in a local and systemic inflammatory response, moreover inadequate recovery and failure to resolve this inflammation and an increase in excessive exercise leads to chronic inflammation and immune system suppression (Brentano & Martins Kruel, 2011; Hackney & Koltun, 2012). The type and pattern of the cytokine response has been suggested to cause the immune-suppression in athletes (Hackney & Koltun, 2012). The production of pro-inflammatory mediators such as TNF-α, IL-1β, IL-6 and prostaglandins are the key mediators of immune-suppression of OTS (Hackney & Koltun, 2012). The production of these mediators causes the suppression of the cell-mediated immunity components of the adaptive immune system thus increasing the risk of illness or illness like symptoms such as upper respiratory symptoms (URS) and infections (URI) impairing physical performance capacity in athletes (Hackney & Koltun, 2012).

Table 2-1: Summary of the role and function of cytokines in response to physical activity and exercise

Cytokine/ acute phase protein	Classification		Main producing cells	Characteristics
	Pro-inflammatory	Anti-inflammatory		
IL-1β	√		Monocytes, macrophages & neutrophils	Release depends on the type of exercise, intensity & a lesser degree duration. Activates the release of TNF-α and IL-6 Promotes the acute phase response Among the first cytokines to be released in response to exercise stress Induces enzymes needed for prostaglandins (PG) & NO synthesis Potent activator of IL-10
IL-2	√		Lymphocytes	Proliferation of T & B lymphocytes Induces potent pro-inflammatory mediator IFN-γ
IL-4		√	Helper T cells, Th2 cells, basophils	Inhibits production of IL-1β, TNF-α & IL-6 Induces differentiation of Th0 to Th2 cells Proliferation & differentiation of B lymphocytes Reduces production of NO and ROS
IL-6	√	√	Monocyte, macrophage & muscle cells	The magnitude of IL-6 response depends on the intensity of the exercise Responsible for the local damage to skeletal muscle Activates respiratory explosion in neutrophils Activates production of CRP during the acute phase response Suppression of IL-1β & TNF-α synthesis by macrophages and neutrophils Activates production of anti-inflammatory cytokines IL-4 & -10 to initiate the resolution of inflammation
IL-10		√	Monocytes & macrophages	Inhibits production of IL-1β, IL-6 & TNF-α Inhibits NO production from macrophages
TNF-α	√		Mononuclear phagocytes, monocytes & macrophages	Influenced by intensity and particularly by the duration of exercise stimulus Induces production of IL-1β Potent activator of IL-10
CRP	√		Hepatocytes	Produced during the acute phase response in response to IL-6 Activates the compliment immune system Promotes phagocytosis by macrophages to clear necrotic an apoptotic cell Plays a role in the innate immunity as early defence system

IL-1β, interleukin-1 beta; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-alpha; CRP, C-reactive protein; IFN-γ, interferon-gamma; NO, nitric oxide; ROS, reactive oxygen species **(Adapted from Moldoveanu *et al.* (2001); Terra *et al.* (2012))**

2.4 Omega-3 polyunsaturated fatty acids role in inflammation

Acute inflammation is part of the body's normal defence mechanism to tissue injury and infection (Serhan & Petasis, 2011). Although the inflammatory response is protective to the body, failing to resolve inflammation can result in chronic and systematic inflammatory disorders (Serhan & Petasis, 2011). Lipid-derived mediators (LDM) play a key role in the initiation and the resolution of inflammation (Serhan *et al.*, 2015b; Serhan & Petasis, 2011). This LDM are synthesized from long-chain polyunsaturated fatty acids (LCPUFAs) that are incorporated in the cell membrane phospholipids (Raphael & Sordillo, 2013).

Cell membranes consist of four main classes known as phospholipids, sphingolipids, glycolipids and cholesterol (Raphael & Sordillo, 2013). The lipid class that is available in large quantities is phospholipids (Raphael & Sordillo, 2013). The cell membrane consists of a lipid bilayer composed mainly out of phospholipids (Raphael & Sordillo, 2013). Phospholipids consist of a hydrophilic head which is facing outwards and hydrophobic tails that are facing inwards on either side of the aqueous regions as shown in **Figure 2-5** (Raphael & Sordillo, 2013). The backbone of phospholipids consists of a glycerol-3-phosphate that contains three adjoining carbon atoms, a polar head group and two fatty acids (Raphael & Sordillo, 2013). The three adjoining carbons are numbered sn-1, sn-2 and sn-3 (Raphael & Sordillo, 2013). Saturated fatty acids are bound to the sn-1 position whereas unsaturated fatty acids are bound to the sn-2 position (Raphael & Sordillo, 2013). LCPUFAs such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are mostly incorporated in phospholipids at the sn-2 position (Calder, 2008). AA, EPA, and DHA are derived from two essential fatty acids namely omega-6 (n-6) and omega-3 (n-3) which are originated from linoleic acid (LA) and α-linolenic acid (ALA), respectively (Calder, 2008).

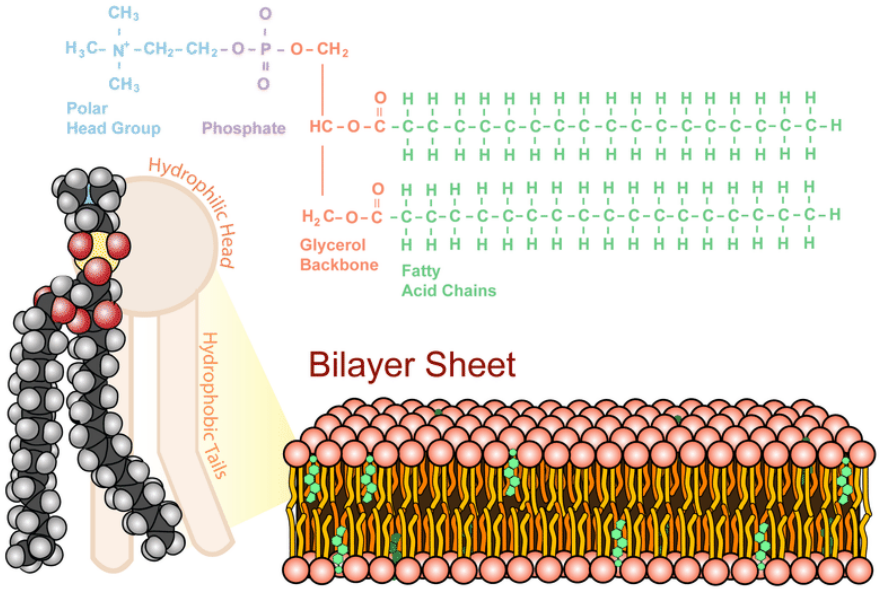


Figure 2-4: The phospholipid within the cell membrane.

The membrane consists of lipid bilayer composed mainly out of phospholipids, with hydrophobic tails facing inwards and hydrophilic heads facing outwards. (Adapted from https://www.ck12.org/book/CK-12-Biology-Concepts/section/2.4/. Date of access: 4 Nov 2016).

Since n-6 and n-3 PUFAs are essential and cannot be produced by the human body, it must be consumed through the diet (Calder, 2006). The most common n-6 and n-3 PUFAs that are consumed by people are LA and ALA, respectively (Raphael & Sordillo, 2013). A typical western diet contains products high in n-6 fatty acids such as soy, corn, sunflower and sunflower oils is the result of an increased LA (n-6 fatty acids) consumption (James *et al.*, 2000; Wood *et al.*, 2014). Therefore, there is a lower intake of ALA (n-3 fatty acids) which is presented in fatty fish, leafy greens, flaxseed, and canola oils (Calder, 2006; James *et al.*, 2000). As shown in **Figure 2-5,** once dietary linoleic acid (n-6 LCPUFA) and alpha-linolenic acid (n-3 LCPUFA) are consumed precursor molecules arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are imbedded in the phospholipid membrane of the immune cells (Calder, 2008). The increased intake of n-6 LCPUFAs, which is pro-inflammatory, through western diet results in a higher amount of AA in the phospholipid membrane (Calder, 2008; James *et al.*, 2000). In contrast, an increased consumption of n-3 PUFAs which is anti-inflammatory will result in an increased amount specifically of EPA and DHA in the phospholipid membrane of the inflammatory cell (Calder, 2006). AA is commonly the dominant substrate in the phospholipid membrane of immune cells (Calder, 2006). Therefore, an increase of EPA and DHA will decrease the amount

of AA in the phospholipid membrane (Calder, 2010). AA, EPA, and DHA are major precursors for pro- and anti-inflammatory LDM (Calder, 2008).

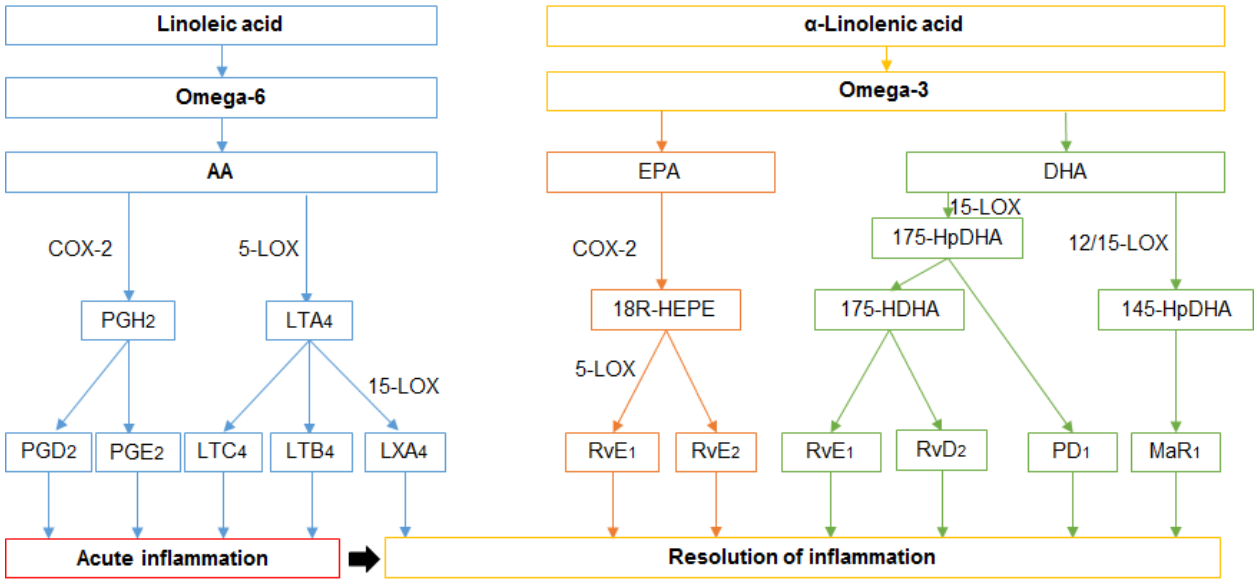


Figure 2-5: Overview of AA, EPA, and DHA-derived lipid mediator synthesis and actions.

AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; COX, cyclooxygenase; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandins; LX, lipoxins; Rvs, resolvins; PDs, protectins; Ma, Marsins (Adapted from Serhan and Petasis (2011)).

Lipid-derived mediators are a family of inflammatory mediators and play a key role in modulating the intensity and duration of inflammatory responses (Calder, 2006; Calder, 2008). These LDM are also known as eicosanoids and play an important role as key mediators and regulators of inflammatory responses to oxidative stress (Sterz *et al.*, 2015). LDM have cell- and stimulus-specific source and frequently have opposing effects (Calder, 2010). As signalling molecules, the lipid-derived mediators are produced and secreted from many different types of cells depending on the function of the signalling molecules (Sterz *et al.*, 2015). AA-derived lipid mediators include prostaglandins (PG), lipoxins (LX), leukotrienes (LT) and hydroxyeicosatetraenoic acids (HETE) (Calder, 2010). Whereas, EPA and DHA give rise to newly discovered resolvins (Rv), protectins (PD) and maresins (MaR) that have resolving qualities (Calder, 2010). These newly discovered lipid mediators are known as specialized pro-resolving lipid mediators (SPM) (Serhan *et al.*, 2015a). As mentioned earlier AA is the dominant substrate in the immune cell phospholipid membranes, therefore they are the major precursors for pro-inflammatory LDM and are produced in high amounts upon cellular stimulation (Calder, 2006). Although AA-derived lipid mediators are

pro-inflammatory it is now recognised that PGE_2 has both pro- and anti-inflammatory qualities and that another lipid mediator derived from AA, lipoxins has anti-inflammatory qualities (Buckley *et al.*, 2014; Calder, 2010). In the phospholipid membrane of the immune cells, AA, EPA, and DHA is stimulated by the phospholipase A_2 enzyme in response to ROS, catalyzing the biosynthesis of pro-inflammatory and anti-inflammatory lipid-derived mediators (Calder, 2006). During the biosynthesis, AA, EPA and DHA get broken down to lipid-derived mediators via the cyclooxygenase enzyme (COX-1 and COX-2) and lipoxygenase (5-LOX, 12-LOX, and 15-LOX) enzyme as shown in **figure 2-6** (Calder, 2006; Markworth *et al.*, 2013). AA-derived lipid mediators are responsible for the activation of acute inflammation (Serhan & Petasis, 2011). Lipoxins is an anti-inflammatory, AA-derived mediator with an important role in leukocyte interactions (Buckley *et al.*, 2014; Fredman & Serhan, 2011; Serhan & Petasis, 2011). Resolvins are known to have potent anti-inflammatory qualities by blocking the production of AA-derived pro-inflammatory lipid mediators and cytokines, respectively (Fredman & Serhan, 2011). DHA-derived mediators include resolvins as mentioned above, protectins and marsins (Serhan & Petasis, 2011). The anti-inflammatory qualities of protectins enhance macrophage efferocytosis of apoptotic PMNs, therefore promoting the resolution process (Kohli & Levy, 2009; Serhan & Petasis, 2011). Another DHA-derived mediator is marsins and are known for their potent pro-resolving and regenerative qualities when produced by macrophages during the inflammatory response (Serhan *et al.*, 2015a). As described above, these lipid-derived mediators are active mediators of different processes such as physiological and pathological processes but are the key link between fatty acids and the inflammatory response (Calder, 2006; Markworth *et al.*, 2013).

2.5 The effect of omega-3 supplementation on inflammation, muscle damage and sport performance

The ingestion of n-3 LCPUFA through the diet or supplementation has shown to have a wide range of biological effects such as the attenuation of pro-inflammatory cytokine formation from neutrophils and monocytes and has potent anti-inflammatory effects by increasing the formation of anti-inflammatory cytokines (Mori & Beilin, 2004). It has been suggested that increasing the supplemental intake of n-3 LCPUFAs could increase the content of EPA and DHA in the phospholipid membrane of cells involved in inflammation (Calder, 2017). As already mention the incorporation of EPA and DHA happens at the expense of AA and it happens in a time- and dose-dependent manner (Browning *et al.*, 2012; Rees *et al.*, 2006). Some possible side effect has been reported with regards to high dosages of n-3 PUFA supplementation such as a fishy aftertaste, nausea, bloating and belching more severe side effects include prolonged bleeding time and elevations in low-density lipoprotein cholesterol (LDL-C) (Covington, 2004). Therefore, a daily intake of > 2 g/day EPA plus DHA is suggested for recreational and competitive athletes to have

an effect on the inflammatory response (Calder, 2017). Since recreational and competitive athletes constantly partake in prolonged intense and/or high-volume eccentric exercise they are more susceptible to acute inflammation (Peake *et al.*, 2005; Suzuki, 2018). Acute inflammation caused by mechanical muscle damage results in delayed onset of muscle soreness (DOMS) (Kim & Lee, 2014). Overtraining however can result in more prolonged inflammation such as overtraining syndrome (OTS) affecting exercise performance (Hackney & Koltun, 2012; Kim & Lee, 2014). Therefore, nutritional strategies to aid in the recovery after prolonged intense and/or high-volume eccentric exercise are being research to help recreational and competitive athletes of which n-3 PUFAs are included.

2.5.1 The effect of n-3 PUFA supplementation on exercise-induced inflammation and muscle damage

Excessive exercise results in exercise induced muscle damage and inflammation that can cause serious reductions in exercise performance capacity (Slattery *et al.*, 2015). Increasing the intake of n-3 PUFA supplementation will increase the amount of biological active EPA and DHA available for the production of anti-inflammatory mediators including cytokines to attenuate pro-inflammatory cytokines (Calder, 2017). Therefore, possibly help in the repair and regeneration of skeletal muscle promoting the resolution of inflammation (Calder, 2010). The production of pro-inflammatory (TNF-α, IL-2, IL-6 and CRP) and anti-inflammatory (IL-4 and IL- 10) cytokines is an important characteristic of the inflammatory response (Peake *et al.*, 2005). Studies have investigated the effect of n-3 PUFA supplementation on pro-inflammatory cytokines in recreational and competitive athletes through measuring of cytokines (TNF-α, IL-2 and IL-6) and acute phase protein, CRP, by using different exercise modalities **(Table 2-2)** (Andrade *et al.*, 2007; Bloomer *et al.*, 2009; Capó *et al.*, 2014; Da Boit *et al.*, 2015; Delfan *et al.*, 2015; Gray *et al.*, 2012; Lenn *et al.*, 2002; Nieman *et al.*, 2009; Radoman *et al.*, 2015; Saiiari & Boyerahmadi, 2014; Santos *et al.*, 2013; Skarpańska-Stejnborn *et al.*, 2010; Toft *et al.*, 2000). Omega-3 PUFA supplementation with dosages between 3.6 – 6 g/d in endurance training protocols has indicated a significant decrease in the cytokine, TNF-α when compared to their placebo counterparts (Bloomer *et al.*, 2009; Delfan *et al.*, 2015; Saiiari & Boyerahmadi, 2014). Delfan *et al.* (2015) found a decrease in TNF-α after an endurance sculling exercise in 22 elite male competitive paddlers supplemented with 1.2 g DHA and 2.4 g EPA for 4 weeks. Similarly, decreased concentrations of TNF-α was detected in supplemented (6 g/d EPA plus DHA) endurance athletes after a marathon run (Saiiari & Boyerahmadi, 2014). Lastly, a randomized cross-over study done on 14 exercise trained men decreased TNF-α after supplementation with 4.4 g/day (EPA/DHA) for 6 weeks after treadmill walk while carrying a weighted pack (60 min) with increased speed and grade every 5 minutes (Bloomer *et al.*, 2009). As previously mentioned, (see Table 2-1) TNF-α production

seems to be influenced by the intensity and particularly the duration of the exercise and generally peaks 60 minutes after EIMD (Moldoveanu *et al.*, 2001). Therefore, indicating that n-3 PUFA supplementation has the ability to decrease production of pro-inflammatory marker TNF-α, although seven studies contradicted this beneficial finding showing no effect of n-3 PUFA supplementation on TNF-α (Andrade *et al.*, 2007; Capó *et al.*, 2014; Lenn *et al.*, 2002; Radoman *et al.*, 2015; Santos *et al.*, 2013; Skarpańska-Stejnborn *et al.*, 2010; Toft *et al.*, 2000). The differing results from the studies could be attributed to the type of exercise protocol, content of supplementation (krill oil vs fish oil), supplementation dosage, type of participants (recreationally active vs competitive athletes) as well as time of sample measurement.

Furthermore, the effect of n-3 PUFA supplementation on pro-inflammatory marker IL-2 demonstrated an increase in 3 studies (Andrade *et al.*, 2007; Da Boit *et al.*, 2015; Gray *et al.*, 2012). The function of IL-2 in exercise-induced inflammation is not well known although it has been suggested to play a key role in cell-mediated immunity and form part of both positive and negative feedback loops in the immune system (Philippou *et al.*, 2012). Andrade *et al.* (2007) demonstrated an increase in IL-2 after the 1.8 g EPA/DHA supplementation over 6 weeks in male competitive swimmers. Similarly, (Gray *et al.*, 2012) supplemented 16 recreational active athletes with 3 g EPA/DHA or 3 g olive oil over a period of 6 weeks and indicated an increase in IL-2 during the recovery period. Furthermore, after the supplementation of 2 g krill oil in 37 male and female participants for 6 weeks, IL-2 was higher in the krill oil group compared to the placebo group (Da Boit *et al.*, 2015). Whereas two studies with dosages between 1.4 – 3 g/d n-3 PUFAs per day did not demonstrate any beneficial effect in football players and marathon runners, respectively (Capó *et al.*, 2014; Santos *et al.*, 2013).

With regards to CRP, four studies indicated a beneficial effect of n-3 PUFA supplementation (Bloomer *et al.*, 2009; Hosseini *et al.*, 2015; Lembke *et al.*, 2014; Santos *et al.*, 2012). During the acute phase response, the pro-inflammatory mediator, CRP, is one of the first mediator to be produced initiated by IL-6 and TNF-α (Kasapis & Thompson, 2005). Bloomer *et al.* (2009) in a cross-over study design indicated decreased CRP concentrations in the experimental group (2.224 g EPA/ 2.208 g DHA) supplemented for 6 weeks after an endurance exercise protocol. A study done on 40 bodybuilding athletes partaking in an overload eccentric exercise protocol demonstrated a decrease in CRP levels in the experimental group after 8 weeks supplementation with 3 g EPA/DHA (Hosseini *et al.*, 2014). Likewise, Lembke *et al.* (2014) also found lower CRP concentration in the experimental group (2.7 g EPA/DHA) compared to the placebo group after multiple sets of maximum eccentric forearm extension. Moreover, in military personnel supplemented (3 g/d EPA plus DHA) for 4 weeks also demonstrated a decrease in CRP (Santos *et al.*, 2012). In disagreement, a study conducted on 20 collegiate male handball players indicated

after 1 week of supplementation of 1 g EPA/DHA no effect on CRP in the experimental group but indicated higher concentrations in the placebo group (Atashak' *et al.*, 2013) indicating that n-3 PUFA supplementation can possibly have a beneficial effect. Furthermore, Nieman *et al.* (2009) found no effect of 2.4 g EPA plus DHA on trained cyclists after an endurance exercise protocol.

Studies reporting on the effect of n-3 PUFA supplementation on anti-inflammatory cytokine IL-10 has not come to a clear conclusion (Capó *et al.*, 2014; Da Boit *et al.*, 2015; Delfan *et al.*, 2015; Santos *et al.*, 2013). An increase in IL-10 was observed in elite paddlers after 3.6 g/d n-3 PUFA supplementation for 4 weeks (Delfan *et al.*, 2015). In contrast, Santos *et al.* (2013) found a decrease in IL-10 after supplementation of 3 g/d in marathon runners. Whereas, no effect was observed in football players and exercise-trained individuals, respectively (Capó *et al.*, 2014; Da Boit *et al.*, 2015). Moreover, no beneficial effect was found for the anti-inflammatory marker IL-4 (Andrade *et al.*, 2007; Capó *et al.*, 2014; Da Boit *et al.*, 2015; Delfan *et al.*, 2015; Gray *et al.*, 2012; Santos *et al.*, 2013).

Since IL-6 is known to have pro-inflammatory (initiates CRP during the acute phase response) and anti-inflammatory (activation of anti-inflammatory cytokines IL-4 and IL-10) properties, the effect of n-3 PUFA supplementation will be difficult to interpret (Moldoveanu *et al.*, 2001). Furthermore, only one study indicated an increase in IL-6 after endurance sculling protocol in competitive paddlers after supplementation of 6 g/d n-3 PUFAs (Delfan *et al.*, 2015) and eight studies indicated no effect of n-3 PUFA supplementation on recreational and competitive athletes (Capó *et al.*, 2014; Da Boit *et al.*, 2015; Gray *et al.*, 2012; Jakeman *et al.*, 2017; Lenn *et al.*, 2002; Nieman *et al.*, 2009; Radoman *et al.*, 2015; Toft *et al.*, 2000).

With regards to the effect of n-3 omega supplementation on muscle damage, Hosseini *et al.* (2014) observed decreased levels of muscle damage marker Creatine Kinase (CK) following 3 g/d omega-3 PUFA supplementation in bodybuilder athletes after an overloading training program. However, in contrast an increase in CK was observed in military personnel and recreational active individuals after supplementation of 3 g/d n-3 PUFAs (Gray *et al.*, 2014; Santos *et al.*, 2012). The increase in CK levels in these two studies could be due to the large eccentric component they used in their exercise protocol. This muscle damage marker is known to be induced during unaccustomed eccentric muscle contraction (Baird *et al.*, 2012).

2.5.2 The effect of n-3 PUFA supplementation on exercise performance and cardiovascular capacity

The possible effect of n-3 PUFA supplementation on exercise performance and capacity has also been explored through different mechanisms (Buckley *et al.*, 2009; Lembke *et al.*, 2014;

Macartney *et al.*, 2014; Peoples *et al.*, 2008; Żebrowska *et al.*, 2015). The mechanisms explored thus far is the effect of n-3 PUFA on the cardiovascular capacity as shown by four studies (Buckley *et al.*, 2009; Macartney *et al.*, 2014; Peoples *et al.*, 2008; Żebrowska *et al.*, 2015). Macartney *et al.* (2014) has demonstrated that a low dose of n-3 PUFA supplementation, 0.56 g/d DHA and 0.14 g/d EPA, administered to 39 physically fit males for 8 weeks reduces submaximal heart rate (HR) during submaximal cycling exercise as well as promotes HR recovery post-exercise. Moreover, Peoples *et al.* (2008) indicated lower HR and HR_{peak} during incremental workloads to exhaustion as well as lowered steady-state submaximal exercise HR, whole body O_2 consumption and rate pressure product (RPP) after the administration of 0.8 g/d EPA and 2.4 g/d DHA for 8 weeks. Żebrowska *et al.* (2015) found that n-3 PUFA administered at a dosage of 0.66 g/d EPA and 0.44 g/d DHA combined for 3 weeks increased VO_2 and endothelial function during a maximal exercise intensity cycling test. Similarly, Buckley *et al.* (2009) indicated a decrease in HR during submaximal exercise in Australian Rules football players after the administration of 0.26 g/d DHA and 0.06 g/d EPA fish oil supplementation. Although above mentioned studies indicated n-3 PUFA supplementation has beneficial effects on cardiovascular capacity, 4 studies indicated no effect on endurance exercise performance (i.e. time trail) (Da Boit *et al.*, 2015; Hingley *et al.*, 2017; Nieman *et al.*, 2009; Oostenbrug *et al.*, 1997). Lewis *et al.* (2015) observed a decrease in time trial performance following 3 weeks of fish oil supplementation compared to a placebo group. Furthermore, no effect was found for time to exhaustion measure for the two studies included in this review (Buckley *et al.*, 2009).

2.6 Conclusion

Intense prolonged and/or eccentric exercise triggers a systematic stress response to skeletal muscle leading to EIMD which is closely regulated by the cellular and molecular components to activate the inflammatory response. The inflammatory response is regulated by the production of pro- and anti-inflammatory cytokines, acute phase proteins and enzymes to repair, restore and regenerate damage to the skeletal muscle. Indeed, the magnitude of the inflammatory response depends on the volume, intensity and duration of the exercise stimulus and the proper function of this system can be impaired during prolonged and/or intense exercise leading to DOMS or in the case of a chronic dysregulation, OTS. The effects of n-3 PUFA supplementation has been suggested to aid in the detrimental effects of the inflammatory response by attenuating the production of pro-inflammatory cytokines and increase the production of anti-inflammatory cytokines. Although a number of studies already observed a beneficial effect of n-3 PUFA supplementation in decreasing pro-inflammatory cytokine TNF-α, there is still a need for high quality studies in this area.

Table 2-2: Summary of effects of n-3 PUFA supplementation on inflammation and muscle damage.

Reference (year), country	Population, gender, n	Intervention		Total n-3 (EPA/DHA) (mg/d)	Duration	Exercise	Effect of n-3 supplementation
		Experimental	Control/ placebo				
Andrade *et al.* (2007), Brazil	Competitive swimmers, male, 20	Fish oil; EPA & DHA	Mineral oil	1800	6 weeks	Swim training protocol	No significant change for IL-4 & TNF-α Significant increase in IL-2 Significant decrease in PGE_2
Atashak *et al.* (2012), Iran	Collegiate handball players, male, 20	Omega-3; EPA & DHA in a base of natural Vit E, gelatine glycerol and purified water	NR	3000	7 days	High intensity acute resistance exercise	No significant change in CK & CRP
Bloomer *et al.* (2009), USA	Exercise-trained individuals, male, 14	EPA & DHA	Soybean oil	4500	6 weeks	Treadmill walk while carrying a weighted pack (60 min) with increased speed and grade every 5 min	Significant decrease in CRP and TNF-α No significant effect on muscle soreness
Capó *et al.* (2014), Spain	Soccer players, male, 15	DHA-rich beverage with 0.6% of olive oil	Beverage with 0.8% of olive oil without DHA	1400	8 weeks	2-hour habitual training program	No significant effect for IL-2, -4, -6 & TNF-α
Da Boit *et al.* (2015), UK	Active individuals, male & female, 37	Krill oil; EPA & DHA with 61µg astaxanthin	Placebo oil was produced to reflect the fatty acid composition of the average European diet	2000	6 weeks	Ergometer exercise trail	Significant increase in IL-2 No effect on IL-4, -10 & -6
Delfan *et al.* (2015), Iran	Competitive paddlers, male, 22	Omega-3; EPA & DHA	Mineral oil	6000	4 weeks	Endurance sculling exercise	No effect for IL-4 Significant increase in IL-6 Significant increase in IL-10 Significant decrease in TNFα

Reference (year), country	Population, gender, n	Intervention		Total n-3 (EPA/DHA) (mg/d)	Duration	Exercise	Effect of n-3 supplementation
		Experimental	Control/ placebo				
Gray *et al.* (2012), UK	Recreational active individuals, male, 16	EPA & DHA with 45I.U. d-α tocopherol	Olive oil	3000	6 weeks	Endurance Wingate exercise	Significant increase in IL-2 No effect for IL-4 & -6
Gray *et al.* (2014), UK	Recreational active individuals, male, 20	EPA & DHA with 45I.U. d-α tocopherol	Olive oil capsules consisted of 0,4 g palmitic acid, 0,08 g stearic acid, 2,1 g oleic acid and 0,37g linoleic acid	3000	6 weeks	Resistance exercise	Significant increase in muscle soreness Significant increase in CK
Hosseini *et al.* (2015), Iran	Bodybuilders , male, 20	Omega-3; EPA & DHA	No supplementation	3000	8 weeks	Overload resistance training	Significant increase in CRP Significant decrease in CK
Jakeman *et al.* (2017), UK	Physically active individuals, male, 18	EPA & DHA	Filler oil, flavour masker and gelatine	1/10 kg	4 days	Plyometric exercise	No significant effect on muscle soreness No effect on IL-6 & CK
Lembke *et al.* (2014), USA	Physically active individuals, female & male, 64	Omega-3 capsules	High oleic sunflower oil capsules	2700	4 weeks	Heavy eccentric exercise	No effect on CK Significant lower levels of CRP
Lenn *et al.* (2002)	Recreational active individuals, male & female, 10	Omega-3 and wheat flour with 100 IU of d-α tocopherol/ dL-α tocopheryl acetate	Wheat flour with 100 IU of d-α tocopherol/ dL-α tocopheryl acetate	1800	4 weeks	Resistance training	No significant effect for TNF-α, CK & IL-6
Nieman *et al.* (2009), USA	Trained cyclists, male, 23	Fish oil concentrate from anchovy and sardines, with soy oil, natural flavours, tocopherols,	Soy oil, natural flavours, tocopherols,	2400	6 weeks	Endurance exercise	No significant effect for CRP, CK & IL-6

Reference (year), country	Population, gender, n	Intervention		Total n-3 (EPA/DHA) (mg/d)	Duration	Exercise	Effect of n-3 supplementation
		Experimental	Control/ placebo				
		canola oil, and citric acid; EPA & DHA	canola oil, and citric acid				
Poprzecki *et al.* (2009), Poland	Moderately trained individuals, male, 24	EPA & DHA with α-tocopherol	Gelatine capsules	1300	6 weeks	Endurance exercise	No effect on CK levels
Radoman *et al.* (2015), Serbia	Soccer players, male, 50	Fish oil; Omega-3	No supplementation	2500	8 weeks	Normal training program	No significant effect for TNF-α & IL-6
Saiiari and Boyerahmadi (2014), Iran	Endurance runners, male, 20	Omega-3; EPA & DHA	Mineral oil	3600	2 weeks	Marathon run	Significant decrease in TNF-α
Santos *et al.* (2012), Brazil	Military personnel, NR, 17	Gelatine coated capsules with EPA & DHA	Maltodextrin	3000	4 weeks	Military regimen	Significant increase in CK Significant decrease in CRP
Santos *et al.* (2013), Brazil	Marathon runners, male, 21	EPA & DHA with α-tocopherol	No supplementation	3000	8 weeks	Marathon run	No significant alterations in IL-2 & TNF-α Decrease in IL-10 IL-4 was not detected after marathon race
Skarpańska-Stejnborn *et al.* (2010), Poland	National rowers, male, 17	Krill oil	Dyed gelatine capsules	1000	6 weeks	Endurance rowing exercise	No significant alterations in CK & TNF-α
Toft *et al.* (2000), Denmark	Endurance trained athletes, male, 20	EPA & DHA with α-tocopherol	No supplementation	3600	6 weeks	Marathon run	No significant effect on IL-6, CK & TNF-α

Table 2-3: Summary of the effect of n-3 PUFA supplementation on cardiovascular capacity and exercise performance

Reference (year), country	Population, gender, n	Total n-3 (mg/d)	Duration	Exercise	Effect of n-3 supplementation
Buckley et al. (2009), Australia	Professional football players, male, 25	6000	5 weeks	Progressive overload training	HR during submaximal exercise ↓ HRpeak ↔ HR recovery ↔ Endurance performance (TTE) ↔ Exercise recovery ↔
Hingley et al. (2017), Australia	Exercise-trained individuals, male, 26	2000	8 weeks	Cycling time trial	Resting oxygen consumption ↔ Steady state VO2 ↔ MVC ↔ Time trail ↔ Oxygen consumption during time trail ↓
Kawabata et al. (2014), Japan	Recreationally active individuals, male, 20	3600	8 weeks	Steady state submaximal exercise test	VO_{2max} during incremental ergometer exercise ↔ VO_2 during steady state submaximal exercise test ↓ HR ↔ Borg scale during incremental exercise and steady state submaximal exercise test ↓
Lewis *et al.* (2015), Canada	Athletes, male,31	5000	21 days	Cycling time trial Back squats Wingate test	MVC force ↔ Rate of force development ↔ Voluntary contraction ↔ Back squat performance ↔ Wingate power drop ↓ in experimental group ↓ in time in the experimental group with regards to time trail

Reference (year), country	Population, gender, n	Total n-3 (mg/d)	Duration	Exercise	Effect of n-3 supplementation
Macartney *et al.* (2014), Australia	Physically fit individuals, males, 26	2000	8 weeks	Steady state cycling test	Resting HR ↔ Submaximal HR ↓ HR recovery ↑
Oostenbrug *et al.* (1997), Amsterdam	cyclists, male, 24	6000	3 weeks	Cycling time trial	VO_{2max} ↔ Lactate concentrations ↔ Time trail ↔
Peoples *et al.* (2008), Australia	Cyclists, male, 16	3200	8 weeks	Submaximal exercise test	HR ↓ HR_{peak} ↓ Whole body O_2 consumption during exercise ↓ Rate pressure product ↓ Time to exhaustion ↔
Żebrowska *et al.* (2015), Poland	Cyclists, male, 17	1300	3 weeks	Incremental ergometer exercise test	VO_{2max} and endothelial function ↑

CHAPTER 3: ARTICLE

Omega-3 supplementation attenuates the pro-inflammatory response and enhances the anti-inflammatory response with no effect on exercise performance in athletes: A systematic review and meta-analysis

Johnine Viljoen[1], Cristian Ricci[1], Lize Havemann-Nel[1]

[1]Centre of Excellence for Nutrition (CEN), North-West University, Potchefstroom, South Africa

johninevljn@gmail.com, Lize.havemannnel@nwu.ac.za, Cristian.ricci@nwu.ac.za

Corresponding author: Johnine Viljoen

johninevljn@gmail.com

Centre of Excellence for Nutrition

Internal box 594

Private bag 6001

North-West University

Potchefstroom

South Africa

2025

Keywords: Omega-3 polyunsaturated fatty acids, inflammation, muscle damage, exercise performance, Athletes, active individuals

Abstract (250 words): Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) may alter the exercise-induced inflammatory response and have immunomodulatory effects in athletes and active individuals, however conclusive evidence is lacking. Therefore, the aim of this systematic review and meta-analysis was to evaluate the effect of n-3 PUFA supplementation on inflammation, muscle damage and exercise performance in athletes and active individuals. Seven electronic databases were searched and 18 randomised controlled trials were included for analysis. Meta-analytical synthesis was performed using a random effect analysis to calculate the effect size of n-3 PUFA supplementation on markers of inflammation (Tumor Necrosis Factor [TNF]- α, Interleukin 2 [IL-2], 6 [IL-6], 4 [IL-4], 10 [IL-10] and C-reactive protein [CRP]), muscle damage (creatine kinase [CK]) and exercise performance (time trial and time to exhaustion). A suggestive trend towards a statistically significant beneficial effect of n-3 PUFA supplementation on anti-inflammatory cytokine IL-10 and pro-inflammatory marker CRP was observed. No effect of n-3 PUFA supplementation on inflammatory markers TNF-α, IL-2, IL-4, IL-6, CRP or exercise performance measurements were observed. In conclusion, the increase in the anti-inflammatory cytokine IL-10 and the reduction of CRP concentrations, suggest that n-3 supplementation has the potential to enhance aspects of the immune system and improve exercise recovery.

Introduction

Similar to other stressors such as disease and trauma, exercise, and particularly high volumes of intensive exercise act as a stress on the body (Slattery, Bentley, & Coutts, 2015). The repetitive contraction of the skeletal muscle during exercise causes mechanical and metabolic disturbances, resulting in exercise-induced muscle damage (EIMD) (Peake et al., 2005; Slattery et al., 2015; Tee, Bosch, & Lambert, 2007). EIMD can result from damage to the muscle structure (e.g. muscle fibre tears) due to mechanical stress as well as from metabolic stress which comprises of exercise-induced oxidative stress and inflammation (Brancaccio, Lippi, & Maffulli, 2010; Panza, Diefenthaeler, & da Silva, 2015). In response to EIMD, multiple cellular and molecular processes are activated to restore the structure and function of skeletal muscle (Philippou, Maridaki, Theos, & Koutsilieris, 2012). These cellular and molecular processes include the productions of enzymes (e.g. creatine kinase), soluble mediators such as C-reactive protein (CRP), as well as pro- and anti-inflammatory cytokines (e.g. Interleukins (IL)) (Andrade, Ribeiro, Bozza, Rosa, & do Carmo, 2007; Bessa et al., 2016; Calder, 2010; Santos et al., 2012). Although the inflammatory response following exercise is essential in response to EIMD, failure to resolve inflammation can lead to delayed onset of muscle soreness (DOMS) (Kanda et al., 2013). Moreover, chronic inflammation can lead to an impaired immunity and ultimately progress to overtraining syndrome (OTS) that negatively affects training, recovery and exercise performance (Hackney & Koltun, 2012; Kanda et al., 2013).

Athletes and active individuals are constantly searching for effective nutritional interventions and strategies, including the intake of nutrients and supplements to enhance recovery and improve exercise performance (Da Boit, Hunter, & Gray, 2017; Moreira et al., 2007). It is acknowledged that omega-3 (n-3) polyunsaturated fatty acids (PUFAs) can play a potential role in the inflammatory response, and may improve post-exercise recovery and exercise performance (Da Boit et al., 2015; Gray, Chappell, Jenkinson, Thies, & Gray, 2014; Hosseini, Peeri, Azarbayjani, & Mateenhomaei, 2015; Jakeman et al., 2017; Lembke, Capodice, Hebert, & Swenson, 2014; Lenn et al., 2002b; Saiiari & Boyerahmadi, 2014). When n-3 PUFAs are consumed, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derived from n-3 PUFAs has been shown to alter the membrane composition, thus resulting in a decreased synthesis of pro-inflammatory mediators, which in turn, increase the synthesis of anti-inflammatory factors derived from EPA and DHA (Markworth et al., 2013; Simopoulos, 2007). Three previous review articles evaluated the effect of n-3 PUFA supplementation on inflammation, muscle damage and exercise performance (Da

Boit et al., 2017; Ochi & Tsuchiya, 2018; Șerban & Răzvan, 2017). Unfortunately, no firm conclusion was drawn since the reviews included studies based on different population groups, such as athletes, sedentary, overweight and obese subjects. Moreover, these reviews included studies with different designs, such as observational and experimental studies, which resulted in unequal sample sizes and methodological heterogeneity (Da Boit et al., 2017; Ochi & Tsuchiya, 2018; Șerban & Răzvan, 2017). Conclusive evidence regarding the effect of n-3 PUFA supplementation on exercise performance, muscle damage and exercise-induced inflammation is therefore warranted. This is therefore relevant to sports nutrition, since n-3 PUFA supplementation can be used as a dietary strategy to attenuate exercise-induced inflammation, improve recovery and potentially improve exercise performance.

Therefore, a systematic review and meta-analysis of randomised controlled trials was performed to evaluate the effect of n-3 PUFA supplementation on markers of inflammation and muscle damage, and exercise performance in recreational and competitive athletes.

Methods

Data source and study selection

The present systematic review and meta-analysis followed the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2010). Eligible papers were identified by searching electronic databases and scanning reference lists of included articles. A literature search was conducted using the ScienceDirect, Academic search premier, SPORTDiscus, CINAHL and COCHRANE databases using the terms "Omega-3 fatty acids," "inflammation," "muscle damage," "exercise performance," "physical activity," "exercise" and "athletes" as keywords. The search was restricted to studies published from 1990 to 1st January 2018. For inclusion, the studies had to fulfil the following criteria: have a randomised placebo-controlled (RCT) design; be written in English and conducted on humans of both sexes; have an oral intake of n-3 PUFA supplementation; report on baseline and post-exercise data of TNF-α, IL-6, IL-2, IL-10, IL-4, CK and/or CRP; be recreational or competitive athletes between the ages of 18 to 35 years. Studies were excluded if subjects were healthy untrained individuals, if allocations to treatments were not randomised, if data for a meta-analysis were not reported and still unavailable after contacting authors, and if studies did not have a placebo-controlled group.

Data extraction and quality assessment

The literature search was independently conducted by two investigators (JV and CR). Study selection and data extraction were undertaken by the same two independent investigators. Any disagreements were resolved by consensus and if no agreement could be reached, a third author (LHN) was consulted. Data extraction was conducted by using a pre-piloted electronic data extraction sheet. The Consolidated Standards of Reporting Trials (CONSORT) 2010 checklist was used to assess the quality and compliance of the included studies (Moher et al., 2012). The CONSORT checklist consists of 37 items that could be categorised as "yes" if item was clearly and adequately reported, or "no" if it was partially reported, unclear or not reported at all. Each "yes" answer received a score of "1" and each "no" answer was scored as "0." The CONSORT checklist was scored for each study potentially eligible for inclusion, a score of 24 or higher out of 37 was included into the systematic review and meta-analysis.

Statistical methods

Individual analyses were performed for each inflammatory marker (CRP, TNF-α, IL-6, IL-2, IL-4 and IL-10) and for the muscle damage marker (CK). The mean changes and corresponding standard deviations (SD) from baseline to post-exercise stress were used in data analysis. If a study reported mean and standard error of mean (SEM), the SEM was converted to SD. A random effects meta-analysis was undertaken because the included RCTs were heterogeneous (different countries; dosage of n-3 PUFA; duration of supplementation; different exercise protocols and time of sample measurements; different level of participation (i.e. recreational or competitive)). Between-study heterogeneity and total variation due to heterogeneity were assessed using Q-test and the I^2 statistic, where an $I^2 \geq 50\%$ was considered an index of relevant heterogeneity. The standardised mean difference (SMD) (effect size) and corresponding 95% confidence intervals (CI) were calculated. Sensitivity analysis was conducted, excluding one study at a time and reporting the upper and lower range of variation of the 95% CI estimates. Publication bias was not assessed using formal bias assessment techniques, such as Egger or Begg's test, because of the few studies included in the meta-analysis, instead Funnel's plot visual inspection was undertaken.

All statistical analyses were performed by STATA 12 (StataCorp, College Station, TX). A type-I error rate of 5% ($\alpha = 0.05$) was considered as statistically significant and all statistical tests were two tailed.

Results

Literature search and characteristics

The process by which the included studies were identified is reported in **Figure 1**. The literature search yielded 1,743 studies, with 10 additional studies identified by reference lists of the included articles. After reading the titles and abstracts, 60 studies were included, of which five articles were excluded because they did not concern the topic, leaving 55 articles to be assessed for eligibility. After the exclusion of seven reviews, 14 original papers not fulfilling the criteria, 13 papers due to unavailable data and two papers not written in English, 18 papers were included in the present study, with a total of 409 participants (Andrade et al., 2007; Bloomer et al., 2009; Buckley et al., 2009; Capó et al., 2014; Da Boit et al., 2015; Delfan et al., 2015; Gray et al., 2012; Hosseini et al., 2015; Jakeman et al., 2017; Lenn et al., 2002a; Lewis et al., 2015; Nieman et al., 2009; Peoples et al., 2008; Poprzecki et al., 2009; Saiiari & Boyerahmadi, 2014; Santos et al., 2013; E. P. Santos et al., 2012; Skarpańska-Stejnborn et al., 2010). Of the 18 studies, 11 randomised control trails (RCTs) were eligible and included for meta-analysis for inflammation (Andrade et al., 2007; Capó et al., 2014; Da Boit et al., 2015; Delfan et al., 2015; Gray et al., 2012; Hosseini et al., 2015; Jakeman et al., 2017; Nieman et al., 2009; Saiiari & Boyerahmadi, 2014; Santos et al., 2013; E. P. Santos et al., 2012) and seven for muscle damage **(Table 1)** (Bloomer et al., 2009; Jakeman et al., 2017; Lenn et al., 2002a; Nieman et al., 2009; Poprzecki et al., 2009; E. P. Santos et al., 2012; Skarpańska-Stejnborn et al., 2010). Five studies were included for exercise performance, of which three evaluated time trial performance (Da Boit et al., 2015; Lewis et al., 2015; Skarpańska-Stejnborn et al., 2010) and two studies considered time to exhaustion performance as an outcome **(Table 2)** (Buckley et al., 2009; Peoples et al., 2008).

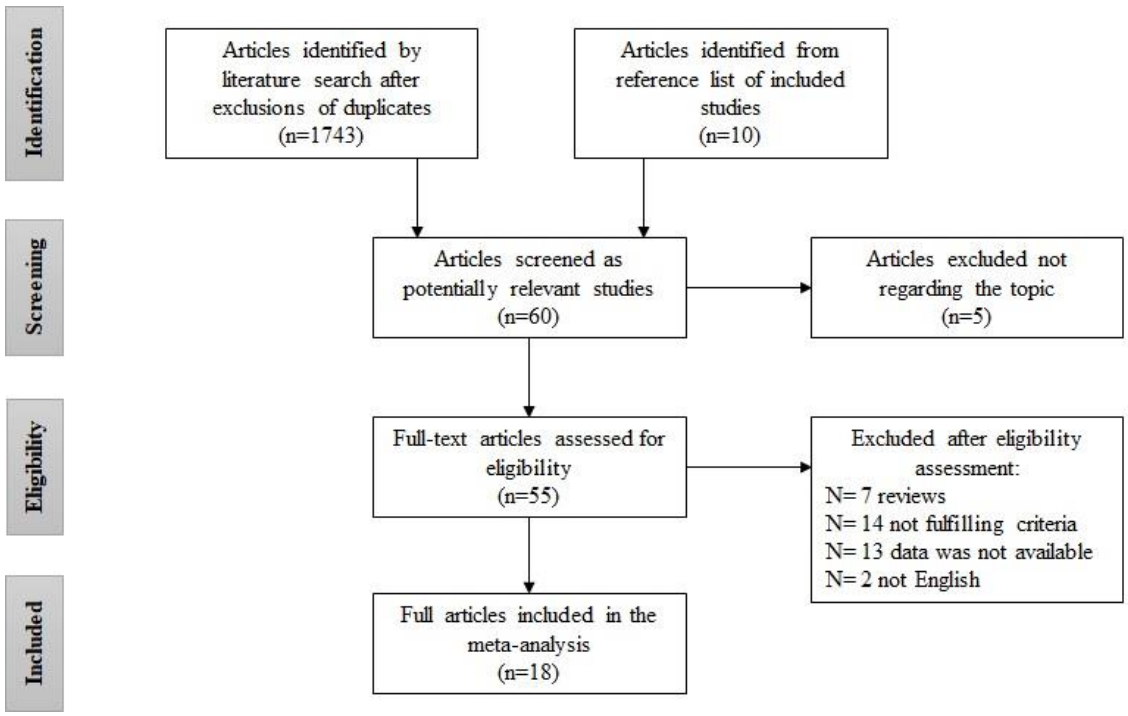


Figure 1. Flow diagram of paper selection. The diagram includes article identification, screening, eligibility, and final inclusion.

119 **Table 1. Studies of included randomised placebo-controlled trails examining inflammatory and muscle damage markers**

Author (year), country	Subjects, gender	No. of subjects (n)		EPA (g)	DHA (g)	Total n-3 (g/d)	Placebo/ Control	Duration	Type of exercise	Biomarkers measured	Time point of blood collection (h)							
		n-3	C								Pre-supp	Post-supp	0 – 30 (min)	1-3	12-24	48	72	96
Andrade et al. (2007), Brazil	Swimmers, male	10	10	0.95	0.50	2.5	P	6 weeks	Swim training	IL-4 & TNF-α	X		X					
Bloomer al. (2009), Memphis	Exercised-trained, male	14	14	2.224	2.208	4.5	P	6 weeks	Aerobic exercise	CK	X		X		X	X		
Capó et al. (2014), Spain	Football players, male	9	6	NR	1.4	1.4	P	8 weeks	Football training	IL-2, IL-4, IL-6, IL-10 & TNF-α	X	X		X				
Da Boit et al. (2015), United Kingdom	Active individuals, male & female	18	19	0.24	0.12	2*	P	6 weeks	Endurance exercise	IL-2 & IL-10	X	X		X				
Delfan et al. (2015), Iran	Elite paddlers, male	11	11	4.8	2.4	6	P	4 weeks	Endurance rowing	IL-4, IL-6, IL-10 & TNF-α	X	X				X		
Gray et al. (2012), United Kingdom	Recreationally active, male	8	8	1.3	0.3	3	P	6 weeks	Endurance exercise	IL-2	X	X		X				
Hosseini et al. (2015), Iran	Bodybuilders, male	10	10	1.2	1.2	3	C	8 weeks	Resistance exercise	CK & CRP	X	X		X				
Jakeman et al. (2017),	Physically active, male	9	9	0.75	0.05	1/10 kg	P	4 days	Plyometric exercise	IL-6 & CK	X			X	X	X	X	X

United Kingdom																		
Lenn et al. (2002a), Kentucky	Recreationally active, male and female	5	5	NR	NR	1.8	P	30 days	Resistance exercise	CK	X	X		X	X	X	X	
Nieman et al. (2009), North Carolina	Cyclists, male	11	12	2	0.4	2.4	P	6 weeks	Endurance exercise	CK & CRP	X	X	X		X			
Poprzeckiet al. (2009), Poland	Moderately trained, male	12	12	0.39	0.26	1.3	P	6 weeks	Endurance exercise	CK		X	X	X				
Saiiari and Boyerahmadi (2014), Iran	Endurance runners, male	10	10	1.2	2.4	3.6	P	2 weeks	Endurance exercise	TNF-α	X	X			X			
Santos et al. (2012), Brazil	Military personnel, NR	9	8	0.54	0.36	3	P	4 weeks	Military regimen	CK & CRP	X	X	X					
Santos et al. (2013), Brazil	Marathon runners, male	8	13	0.3	1.5	3	C	8 weeks	Marathon	IL-2, IL-10 & TNF-α	X		X					
Skarpańska-Stejnborn et al (2010), Poland	Rowers, male	9	8	NR	NR	1*	P	6 weeks	Endurance exercise	CK	X	X	X		X			

n, number of participants; n-3, Omega-3; C, intervention in control/placebo group; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; g, grams; g/d, grams per day; NR, not recorded; IL, interleukins; TNF-α, tumor necrosis factor-alpha; h, hours; min, minutes

* krill oil supplementation

Table 2. Studies of included randomised placebo-controlled trail examining exercise performance measures.

Author (year), country	Subjects, gender	No. of subjects (n)		EPA (g)	DHA (g)	Total n-3 (g/d)	Placebo/ Control	Duration	Type of exercise	Performance measurement
		n-3	Control							
Buckley et al (2009), Australia	Football players, male	12	13	0.36	1.56	6	P	5 weeks	Treadmill run	TTE
Da Boit et al. (2015), United Kingdom	Active individuals, male & female	18	19	0.24	0.12	2*	P	6 weeks	Endurance exercise	TT
Lewis et al (2015), Canada	Athletes, male	18	12	0.375	0.51	5	P	3 weeks	Cycling	TT
Peoples et al (2008), Australia	Cyclists, male	9	7	0.8	2.4	3.2	P	8 weeks	Cycling	TTE
Skarpańska-Stejnborn et al. (2010), Poland	Rowers, male	9	8	NR	NR	1*	P	6 weeks	Endurance exercise	TT

n, number of participants; n-3, Omega-3; C, intervention in control/placebo group; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; NR, not recorded; TT, time trail; TTE, time to exhaustion

* krill oil supplementation

Risk of bias

The Cochrane Collaboration Tool was used to judge the risk of bias among included studies (Higgins et al., 2011). Risk of bias was deemed high if any of the elements assessed were unclear or not reported. Where assessed areas were adequately reported the risk was considered low. The risk of bias for the included studies **(Figure 2)** was found to be high in nine studies (Andrade *et al.* 2007; Capó *et al.*, 2014; Hosseini *et al.*, 2018; Lenn *et al.*, 2002; Nieman *et al.*, 2009; Poprezecki *et al.*, 2009; Saiiari & Boyerahmadi, 2014; Santos *et al.*, 2012 Santos *et al.*, 2013). This was mostly attributed to the lack of information, which made the risk of bias assessment difficult.

The quality of the included studies was evaluated by using the CONSORT checklist. It was found that the quality of included studies was very low due to a lack of reported information.

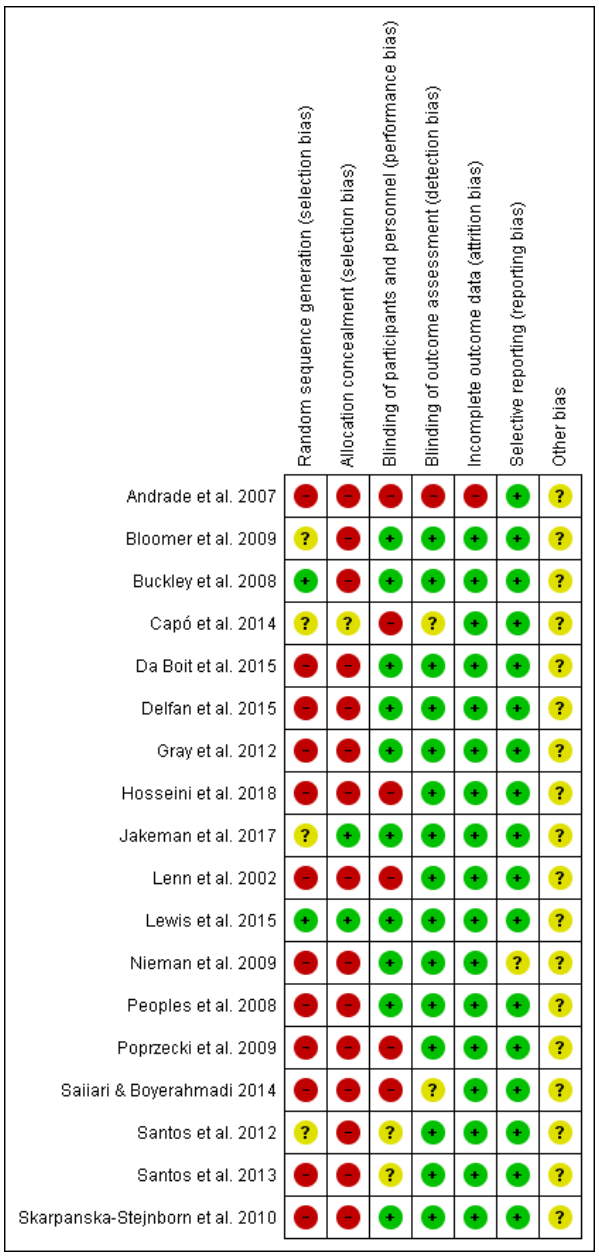


Figure 2. Risk of bias of included studies for inflammation, muscle damage and exercise performance

Effect of n-3 PUFA supplementation on inflammatory markers.

We observed a suggestive trend towards a statistically significant beneficial effect of n-3 PUFA supplementation on anti-inflammatory cytokine IL-10 (SMD = 0.74, 95% CI: -0.08 to 1.56; P = 0.075), with relatively high heterogeneity among the four included studies (I^2 = 70.4%, P = 0.017) **(Figure 3)**. In contrast to IL-10, no significant effect of n-3 PUFA supplementation was observed for IL-4 (SMD = -0.05, 95% CI: -0.58 to 0.47, P = 0.841) and no heterogeneity was observed among these included studies (I^2 = 0.0%, P = 0.593) **(Figure 4)**. Sensitivity analysis, excluding one study at a time, did not show any relevant change in the pooled effect size (ES) and 95% CI for both IL-10 and IL-4. Three studies were included for the analysis of the effect of n-3 PUFA supplementation on acute phase protein CRP (Hosseini et al., 2015; Nieman et al., 2009; E. P. Santos et al., 2012) **(Figure 5)**. In this analysis, we reported a suggestive trend to a statistically significant CRP reduction for subjects having n-3 PUFA supplementation with respect to the placebo (SMD = -2.03, 95% CI: -4.31 to 0.25, P = 0.081) with high heterogeneity among the included studies (I^2 = 91.7%, P < 0.001). Sensitivity analysis, omitting one study at a time, did not show any change for CRP. Omega-3 PUFA supplementation did not show any significant effects for the marker's TNF-α, IL-6 and IL-2 with pooled effects (SMD = 0.38, 95% CI: -1.55 to 0.80; SMD = 0.84, 95% CI: -0.26 to 1.94; SMD = 0.36, 95% CI: -0.36 to 1.8, respectively). Significant heterogeneity was observed among the three groups of studies with the I^2 values of 85.5% (P < 0.001), 72.5% (P = 0.026) and 60.5% (P = 0.054), respectively.

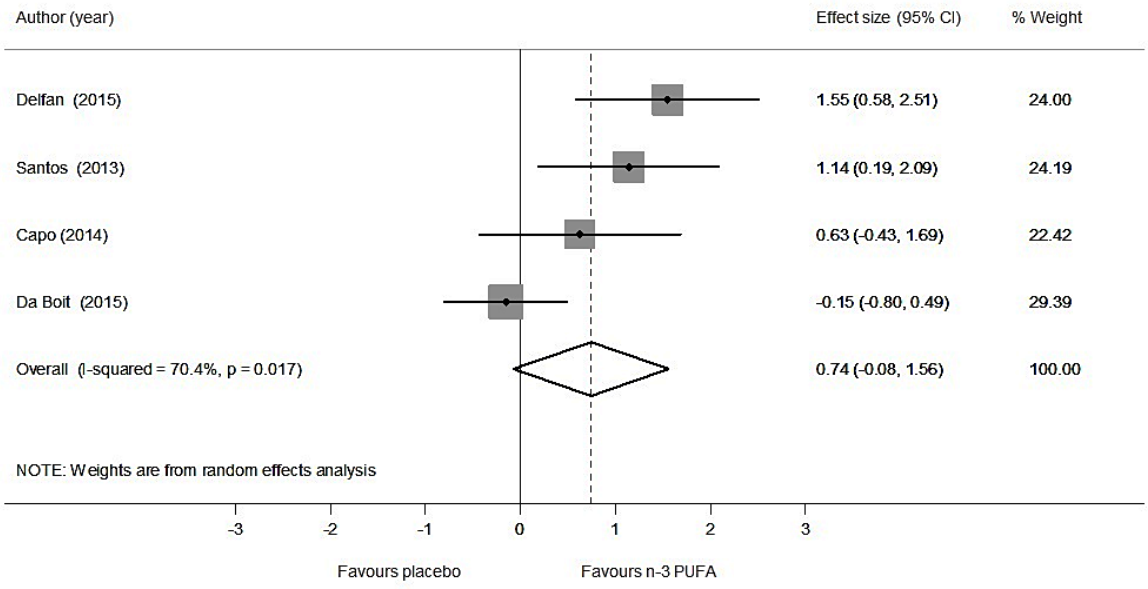


Figure 3. Pooled effect size of n-3 PUFA supplementation on anti-inflammatory cytokine IL-10

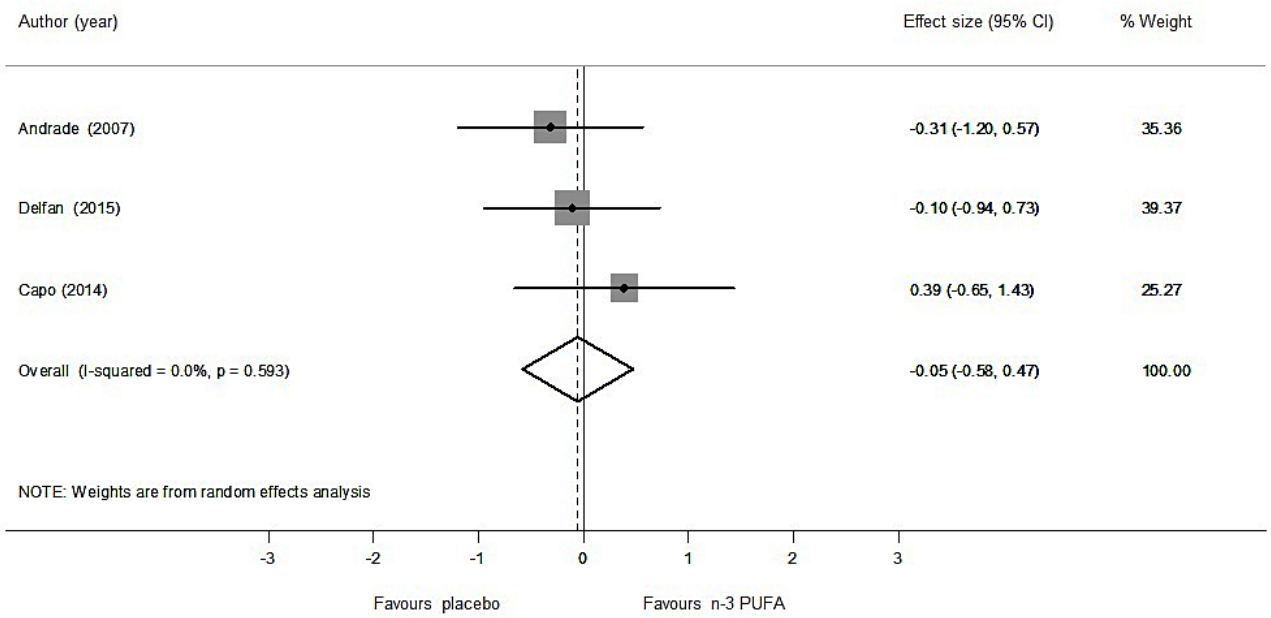


Figure 4. Pooled effect size of n-3 PUFA supplementation on anti-inflammatory cytokine IL-4.

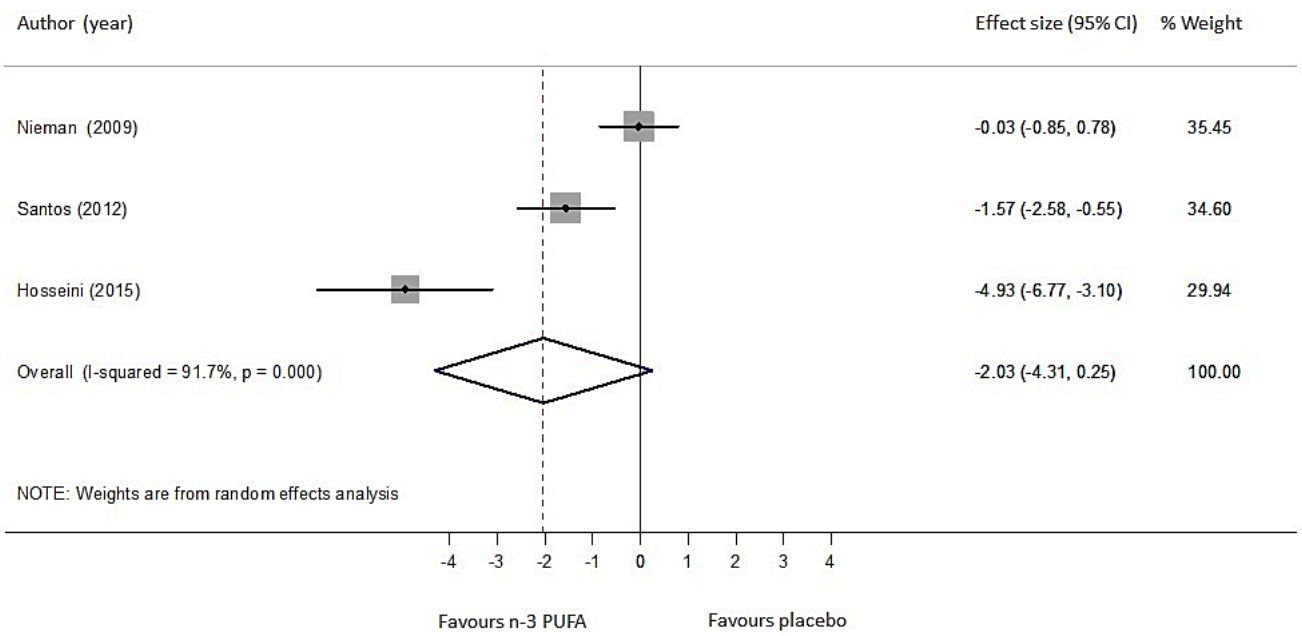


Figure 5. Pooled effect size of n-3 PUFA supplementation on CRP.

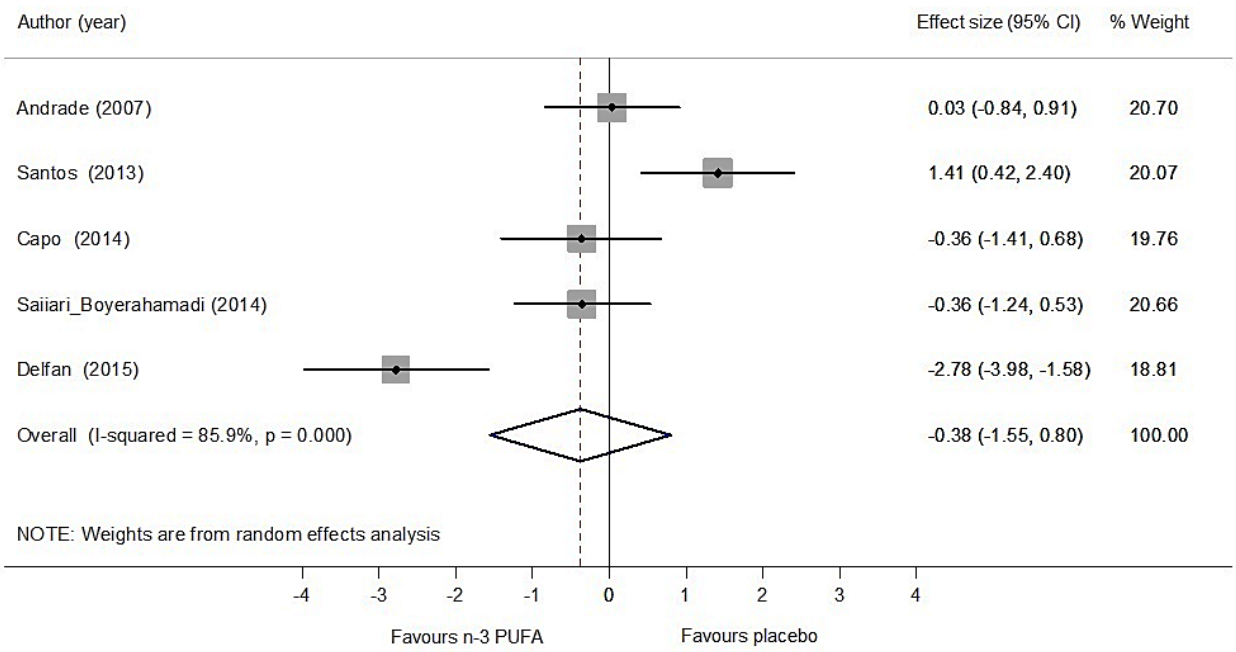


Figure 6. Pooled effect size of n-3 PUFA supplementation on TNF-α.

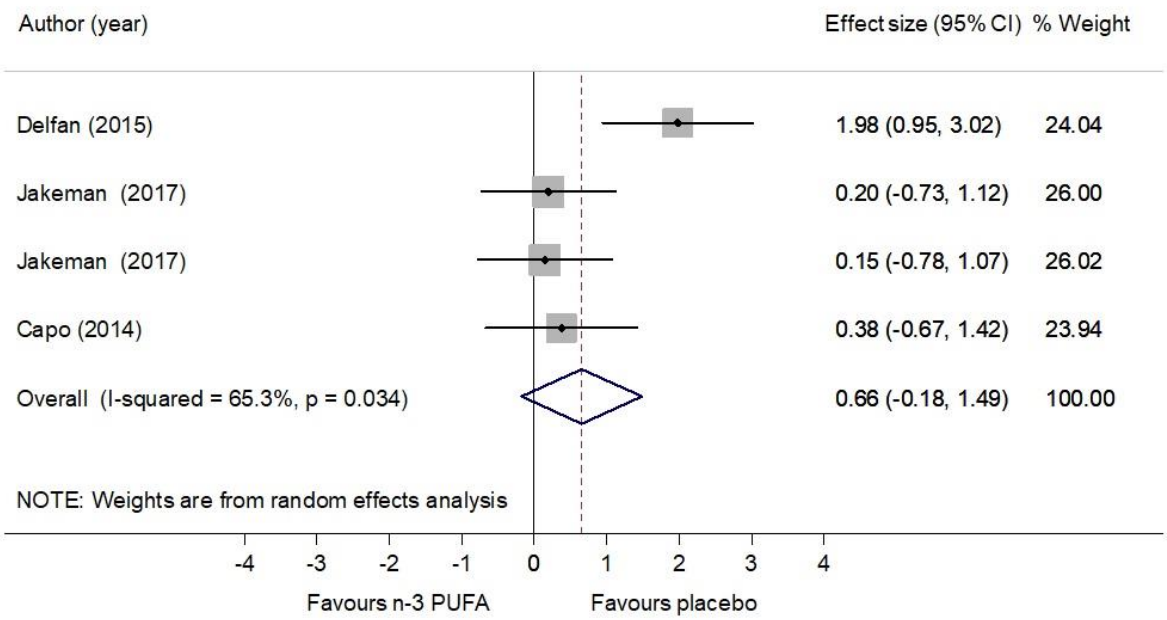


Figure 7. Pooled effect size of n-3 PUFA supplementation on IL-6

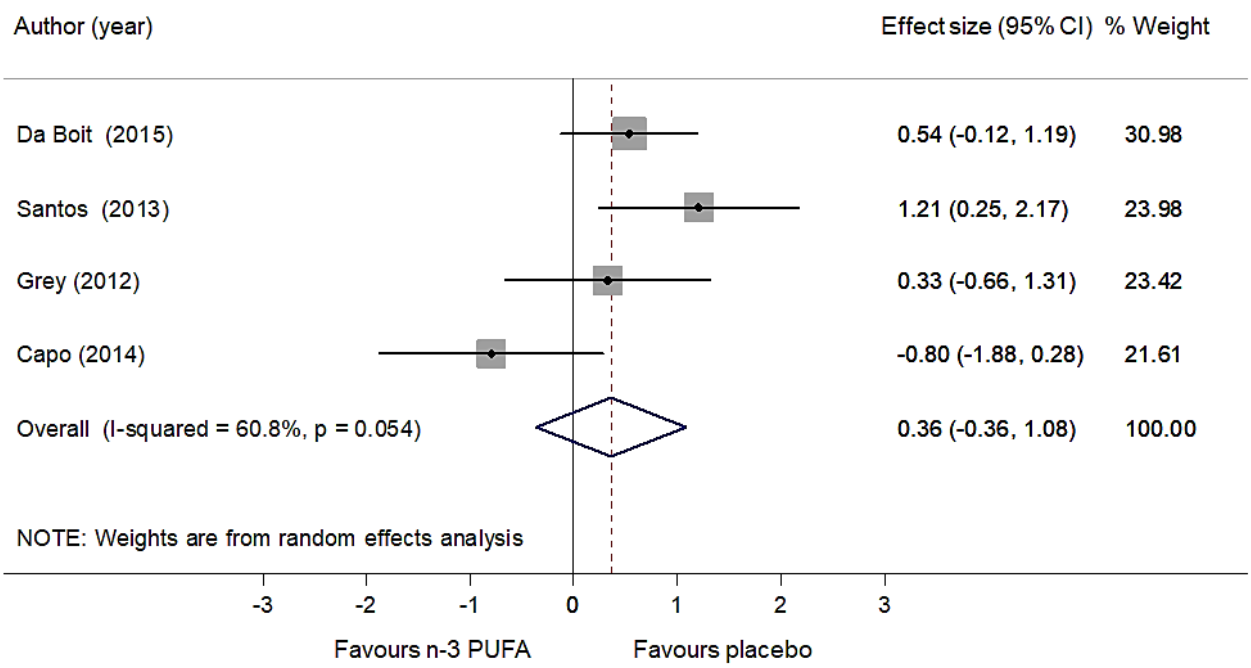


Figure 8. Pooled effect size of n-3 PUFA supplementation on IL-2.

Effect of n-3 PUFA supplementation on muscle damage marker.

Seven studies were included for the analysis of muscle damage marker CK (Bloomer et al., 2009; Hosseini et al., 2015; Jakeman et al., 2017; Nieman et al., 2009; Poprzecki et al., 2009; Santos et al., 2013; Skarpańska-Stejnborn et al., 2010). A non-significant reduction was observed for CK (SMD = -0.39, 95% CI: -0.88 to 0.11, P = 0.123) with moderate, borderline significant heterogeneity (I^2 = 54.3%, P = 0.041) (Figure 9). Exclusion of one study at a time did not show any relevant change of the ES estimate, with SMD ranging from -0.21 (95% CI: -0.62 to 0.21) to -0.53 (95% CI: -1.02 to -0.04).

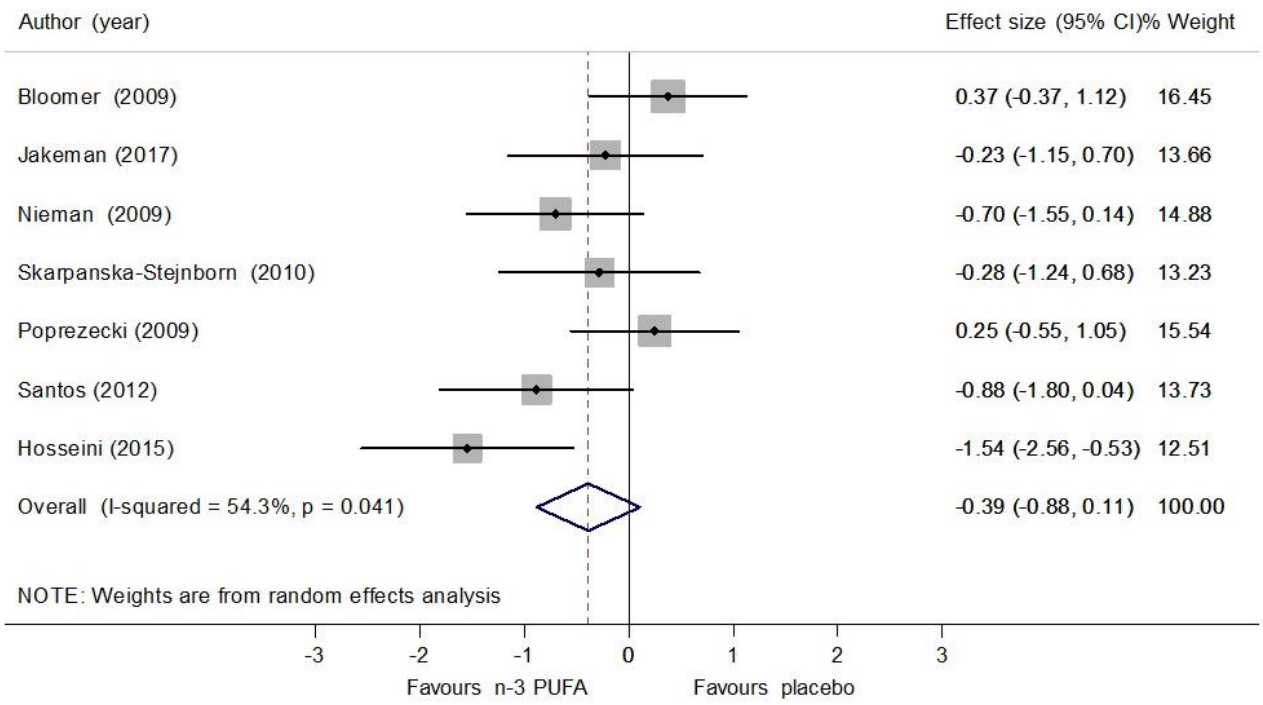
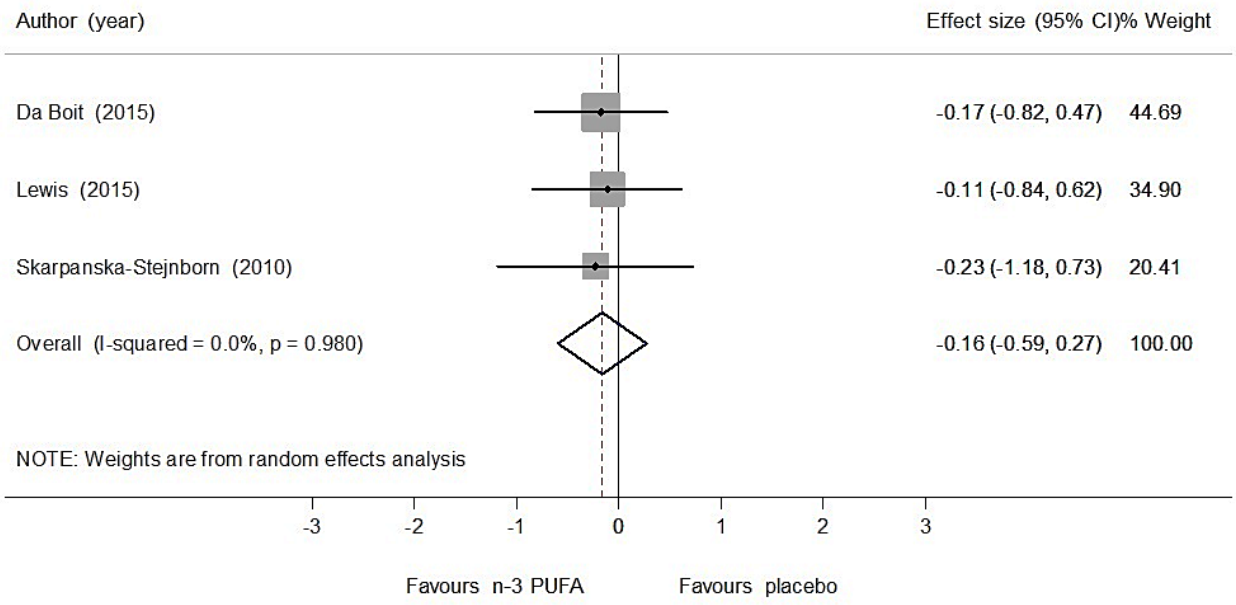


Figure 9. Pooled effect size of n-3 PUFA supplementation on CK.

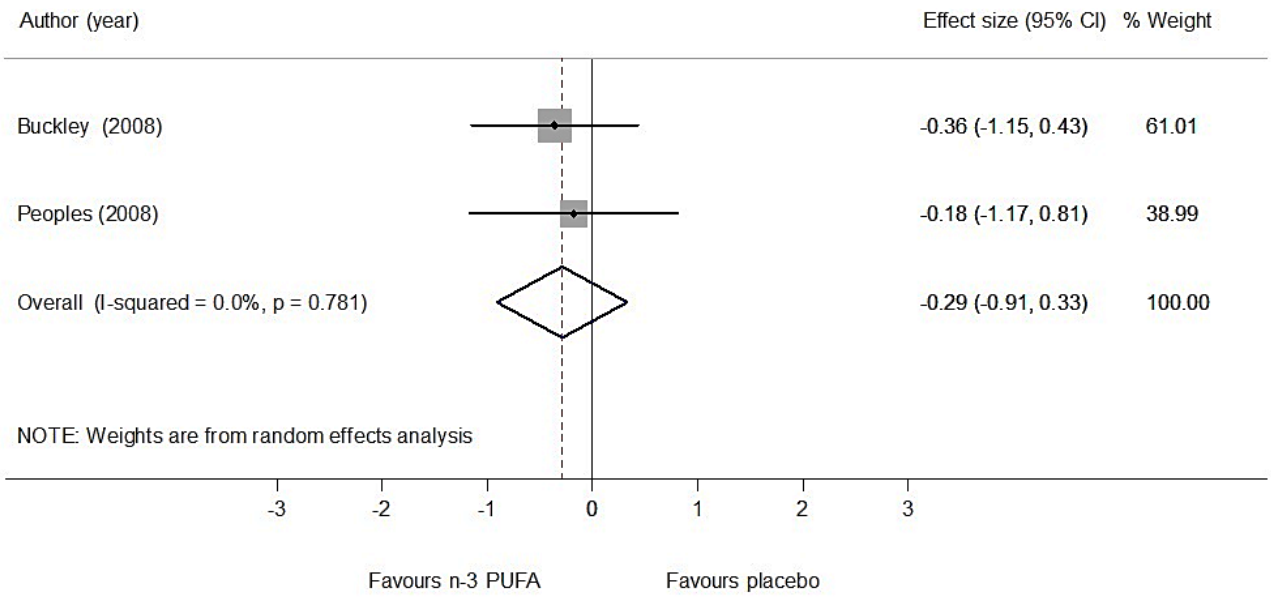
Effect of n-3 PUFA supplementation on exercise performance.

Based on five studies and 125 endurance trained individuals, the random effects meta-analysis did not indicate any significant effect of n-3 PUFA supplementation on exercise performance measures in terms of time trail and time to exhaustion (Buckley et al., 2009; Da Boit et al., 2015; Lewis et al., 2015; Peoples et al., 2008; Skarpańska-Stejnborn et al., 2010). Three studies were included for the time trail analysis (SMD = -0.16, 95% CI: -0.59 to 0.27, P = 0.464, I^2 = 0.0%, P = 0.980) and two studies for time to exhaustion (SMD = -0.29, 95% CI: -0.91 to 0.33, P = 0.358), with no observed heterogeneity (I^2 = 0.0%, P = 0.781).



223

224 **Figure 10**. Pooled effect size of n-3 PUFA supplementation on time trail performance.



225

226 **Figure 11**. Pooled effect size of n-3 PUFA supplementation on time to exhaustion performance.

227

228 *Publication bias*

229 The Funnel plot visual inspection of all the analyses performed revealed a certain degree of
230 symmetry so that the presence of publication bias could be considered as negligible.

Discussion

In the present study, we illustrated a suggestive beneficial effect of n-3 PUFA supplementation on inflammation and recovery. In particular, we reported a suggestive trend towards a statistically significant beneficial effect of n-3 PUFA supplementation on anti-inflammatory cytokine IL-10 and pro-inflammatory marker CRP, although the heterogeneity among these studies were high for the specific markers. However, no difference was demonstrated for muscle damage marker creatine kinase (CK), or for the inflammatory markers IL-4, IL-2, IL-6 and TNF-α. Omega-3 PUFA supplementation also did not improve exercise performance measured as time to exhaustion and time to complete a set task (time trials).

A beneficial effect for n-3 PUFA supplementation to reduce inflammation and muscle damage had previously been reported in scientific studies conducted in untrained subjects (Lembke et al., 2014; Li, Huang, Zheng, Wu, & Li, 2014; Tartibian, Maleki, & Abbasi, 2011). Although the present results are a possible indication that n-3 PUFA supplementation might be an effective strategy to reduce inflammation and exercise-induced muscle damage (EIMD) in recreational and competitive athletes, more studies should be done to come to a clear conclusion. There was no beneficial effect of n-3 PUFA supplementation was not for all of the biomarkers considered, despite the existence of supporting evidence in the literature. The scientific evidence regarding the effect of n-3 PUFA supplementation on IL-2, IL-4 and TNF-α is contradictory (Jaudszus et al., 2013; Li et al., 2014; Toft et al., 2000), which could be attributed to a certain degree of heterogeneity among included studies, in particular a high degree of heterogeneity considering IL-2 and TNF-α as outcomes.

From the current meta-analysis, only one study favoured 8 weeks of n-3 supplementation to decrease pro-inflammatory marker IL-2 in combination with 2-hour habitual training in male football players (Capo et al., 2014). In contrast, three studies showed an unexpected favourable response for the placebo to increase IL-2 in active and recreational individuals and male marathon runners (Da Boit et al., 2015; Gray et al., 2012; Santos et al., 2013) **(Figure 8).** Amongst these three studies, those from Da Boit *et al*., (2015) and Gray *et al*., (2012) included an intense endurance training protocol that was undertaken by active individuals generally involved in recreational physical activity. The study by Santos *et al.,* (2013) included endurance runners supplemented with 3 g EPA plus DHA for 8 weeks with a marathon run as intervention protocol (Santos et al., 2013). It is not clear why there was an unexpected increased IL-2 in response to exercise-induced stress in these studies. One possible explanation could be related to upper respiratory tract infection (URTI), a condition commonly found after prolonged and/or intense

exercise (Martin, Pence, & Woods, 2009). The increased IL-2 in these studies could be an indication of improved antiviral defence since IL-2 promotes the maturation of antigen specific cytotoxic T-lymphocytes which induces cytotoxic killing of virally infected cells, thus, providing some protection against URTI's (Martin et al., 2009). Furthermore, the characteristics of the studies, including sample size and risk of bias, varied widely possibly influencing the results.

In the analysis of anti-inflammatory marker IL-4 only the study of Capo *et al.,* (2014) indicated a favourable increase in IL-4 after 8 weeks of n-3 PUFA supplementation. Whereas no favourable effects of n-3 PUFA supplementation was found in the studies of Andrade *et al*., (2007) and Delfan *et al.,* (2015) in response to intense swimming and paddling protocols, respectively. The lack of an increase in IL-4 in response to n-3 PUFA supplementation could be mostly attributed to the study of Andrade *et al*., (2007). Notably, this study was based on swimmers for which a lower level of inflammation and muscle damage is expected because these athletes are subjected to an un-gravitational environment without important eccentric contraction of the muscles. Furthermore, the risk of bias in the study of Andrade *et al*., (2007) was found to be high, therefore making it difficult to draw a meaningful conclusion on the effect of n-3 PUFA supplementation on IL-4.

Pro-inflammatory marker TNF-α was also not significantly influenced by n-3 PUFA supplementation. A favourable effect of n-3 PUFA supplementation was observed in the studies of Capo *et al.,* (2014); Delfan *et al.,* (2015) and Saiiari & Boyerahmadi *et al.,* (2014) whereas no effect of n-3 PUFA supplementation was observed for the study of Andrade *et al*., (2007). The study of Santos *et al*., (2013) in particular favoured the placebo and the expected decrease in TNF-α in response to n-3 supplementation was not observed. This could be due to two reasons. Firstly, as already mentioned, this study was conducted in marathon runners where the benefits of n-3 PUFA supplementation could be to a lesser extent since this marker in particular is influenced by the duration of the exercise bout and a potent mediator during the initiation of the inflammatory response. Secondly, it should be noted that TNF-α was measured immediately after the exercise bout in this study; since TNF-α is one of the mediators responsible for the initiation of the inflammatory response, the time of measurement of this marker should be taken into account (Suzuki, 2018).

There were no beneficial effects observed for the pro- and anti-inflammatory marker IL-6. This particular inflammatory marker is known to have pro- and anti-inflammatory properties depending on the phase of the inflammatory response and time of cytokine measurement. A total of three studies were included for the analysis of IL-6 where two studies indicated an increase (Jakeman et

al., 2017; Delfan et al., 2015) and one study a decrease (Da Boit et al., 2015). However, the magnitude of IL-6 response is dependent on the intensity of the exercise and also rises due to direct damage to the muscle. Delfan *et al.,* (2015) observed an increase in IL-6 after 4 weeks of n-3 PUFA supplementation in football players in response to 2-hour habitual training. Likewise, the study done by Jakeman *et al.,* (2017) after a high dose of 1g/10kg per day (0.75 g EPA plus 0.05 g DHA) for only four days after plyometric exercise protocol; however, EPA and DHA is incorporated into the phospholipid membrane in a time-dependent matter making 4 days of supplementation a too short period for n-3 PUFA supplementation to have an effect.

With regards to the muscle damage marker CK, no effect of n-3 PUFA supplementation was observed. Seven studies were included for the muscle damage marker, where Bloomer *et al.,* (2009) and Poprezecki *et al.,* (2009) indicated a favourable increase for the placebo in response to an aerobic and endurance exercise protocol, respectively. Since CK is important for the regeneration of cellular ATP, this marker increases in response to a high energy demand therefore the exercise protocol of the above-mentioned studies could be a possible explanation for the increase in CK.

The beneficial effect of n-3 PUFA supplementation was also not confirmed by our meta-analysis when considering exercise performance, which could be due to a number of possible reasons. Firstly, only three of the included studies evaluated time trial performance and two evaluated time to exhaustion which reduces the meta-analysis ability to detect significant effects. Secondly, it is well known that the improvement of exercise performance dependent on muscle strength and endurance as well as cardiovascular capacity and not on n-3 PUFA supplementation duration. Thirdly, not only the treatment duration, but also the n-3 PUFA form of supplementation and dose may have a role in determining exercise performance improvement (Calder, 2015). When looking at the studies from Da Boit *et al.,* (2015) and Skarpańska-Stejnborn *et al.,* (2010) evaluating time trail performance, it was noticed that the n-3 PUFA supplementation dose was particularly low and provided in the form of krill oil.

There was a suggestive beneficial effect of n-3 PUFA supplementation on anti-inflammatory marker IL-10. The increase in IL-10 was indicated by three studies with dosage ranges from 1.4-6 g/d administered for 6-8 weeks in football players, elite paddlers and marathon runners, respectively (Capó *et al.*, 2014; Delfan *et al.*, 2015; Santos *et al.*, 2013). In contrast, Da Boit *et al.*, (2015) did not indicate this beneficial effect this could be due to the administration of krill oil

instead of fish oil. The content of EPA and DHA in Krill oil in this particular supplementation were low, 0.24 g and 0.12 g, respectively, explaining the possible lack of effect on IL-10.

A beneficial effect of n-3 PUFA supplementation was also observed for the pro-inflammatory marker CRP. Moreover, a decrease was observed in the individual studies of Hosseini *et al.,* (2015) and Santos *et al.,* (2012) after the administration of 3 g of n-3 PUFA supplementation for 4-8 weeks in bodybuilders and military personnel. Nieman *et al.* (2009) on the other hand did not show any effect of 2.4 g EPA plus DHA in male cyclists.

The present evidence regarding the beneficial effect of n-3 PUFA supplementation on inflammation, in particular an increase in anti-inflammatory cytokine IL-10 and a decrease in pro-inflammatory marker CRP, has a mechanistic rationale. It is well known that the production of IL-10 are predominantly dependent on the exercise intensity and not exercise-induced muscle damage (Suzuki, 2018). The anti-inflammatory marker IL-10 is activated upon stimulation of IL-6 during the resolution phase of the inflammatory response (Philippou et al., 2012). In return, IL-10 inhibits the production of pro-inflammatory cytokines, such as TNF-α and IL-1β, thus promoting the resolution of inflammation (Calder, 2015; Suzuki, 2018). C-reactive protein is secreted upon the activation of the acute phase response, therefore, a decrease in this marker could be indicative of a decrease in the magnitude of the inflammatory response (Philippou et al., 2012).

The present work has some remarkable strength points. Firstly, it is based on a robust methodology applied to current and comprehensive evidence regarding n-3 PUFA supplementation on recreational and competitive athletes. In addition, an up to date and critical evaluation about the most common conditions under which the effect of n-3 PUFA supplementation is evaluated using RCT was provided. Naturally, the present work had certain weak points as well. For example, a high degree of methodological heterogeneity and risk of bias among studies was reported, the included studies were based on small sample sizes with limited duration and most of the studies did not provide power calculation to justify the sample size considered. On the other hand, these limiting factors provide useful suggestions to scientists involved in randomised controlled studies aimed at assessing the beneficial effects of n-3 PUFA supplementation on athletes and active individuals.

Conclusion

The present systematic review and meta-analysis adds important findings to the scientific literature that could possibly aid scientists, coaches, active individuals and athletes in making evidence-

based decisions regarding the use of n-3 PUFA supplementation to reduce inflammation and muscle damage. The current status of the research on n-3 PUFA supplementation in athletes was also illustrated, providing suggestions to those who are interested in planning a new RCT to evaluate the beneficial effect of n-3 PUFA supplementation on inflammation, muscle damage and exercise performance. Therefore, there are a few limitations of the current available RCT's, such as statistical power and sample size, the target to a specific population of athletes, dose and form of n-3 PUFA supplementation, techniques for biomarker assessment, duration of the RCT, along with the specific evaluation of the training schedule, should be taken into account in the phase of study planning to provide robust and useful evidence.

References

Andrade, P. M., Ribeiro, B. G., Bozza, M. T., Rosa, L. F. B. C., & do Carmo, M. G. T. (2007). Effects of the fish-oil supplementation on the immune and inflammatory responses in elite swimmers. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 77*(3), 139-145.

Bessa, A. L., Oliveira, V. N., Agostini, G. G., Oliveira, R. J., Oliveira, A. C., White, G. E., . . . Espindola, F. S. (2016). Exercise intensity and recovery: biomarkers of injury, inflammation, and oxidative stress. *The Journal of Strength & Conditioning Research, 30*(2), 311-319.

Bloomer, R. J., Larson, D. E., Fisher-Wellman, K. H., Galpin, A. J., & Schilling, B. K. (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 Health Dis, 8*, 36. doi:10.1186/1476-511X-8-36

Brancaccio, P., Lippi, G., & Maffulli, N. (2010). Biochemical markers of muscular damage. *Clinical Chemistry and Laboratory Medicine, 48*(6), 757-767.

Buckley, J. D., Burgess, S., Murphy, K. J., & Howe, P. R. (2009). DHA-rich fish oil lowers heart rate during submaximal exercise in elite Australian Rules footballers. *Journal of Science and Medicine in Sport, 12*(4), 503-507.

Calder, P. C. (2010). Omega-3 fatty acids and inflammatory processes. *Nutrients, 2*(3), 355-374. doi:10.3390/nu2030355

Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1851*(4), 469-484.

Capó, X., Martorell, M., Llompart, I., Sureda, A., Tur, J. A., & Pons, A. (2014). Docosahexanoic acid diet supplementation attenuates the peripheral mononuclear cell inflammatory response to exercise following LPS activation. *Cytokine, 69*, 155-164. doi:10.1016/j.cyto.2014.05.026

Da Boit, M., Hunter, A. M., & Gray, S. R. (2017). Fit with good fat? The role of n-3 polyunsaturated fatty acids on exercise performance. *Metabolism, 66*, 45-54.

Da Boit, M., Mastalurova, I., Brazaite, G., McGovern, N., Thompson, K., & Gray, S. R. (2015). The effect of krill oil supplementation on exercise performance and markers of immune function. *PloS one, 10*(9), e0139174.

Delfan, M., Ebrahim, K., Baesi, F., Mirakhori, Z., Ghalamfarsa, G., Bakhshaei, P., . . . Yousefi, M. (2015). The immunomodulatory effects of fish-oil supplementation in elite paddlers: A

pilot randomized double blind placebo-controlled trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), 99*, 35-40.

Gray, P., Chappell, A., Jenkinson, A. M., Thies, F., & Gray, S. R. (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), 206-214. doi:10.1123/ijsnem.2013-0081

Gray, P., Gabriel, B., Thies, F., & Gray, S. R. (2012). Fish oil supplementation augments post-exercise immune function in young males. *Brain, behavior, and immunity, 26*(8), 1265-1272.

Hackney, A. C., & Koltun, K. J. (2012). The immune system and overtraining in athletes: clinical implications. *Acta Clin Croat, 51*(4), 633-641.

Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., . . . Sterne, J. A. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj, 343*, d5928.

Hosseini, A., Peeri, M., Azarbayjani, M. A., & Mateenhomaei, H. (2015). Effect of resistance training and omega3 supplementation on markers of muscle damage and inflammation in trained men. *Indian Journal of Fundamental and Applied Life Sciences, 5*(2), 122-132.

Jakeman, J., Lambrick, D., Wooley, B., Babraj, J., Faulkner, J., Jakeman, J. R., . . . Faulkner, J. A. (2017). Effect of an acute dose of omega-3 fish oil following exercise-induced muscle damage. *European journal of applied physiology, 117*(3), 575-582. doi:10.1007/s00421-017-3543-y

Jaudszus, A., Gruen, M., Watzl, B., Ness, C., Roth, A., Lochner, A., Jahreis, G. (2013). Evaluation of suppressive and pro-resolving effects of EPA and DHA in human primary monocytes and T-helper cells. *Journal of lipid research*, jlr. P031260.

Kanda, K., Sugama, K., Hayashida, H., Sakuma, J., Kawakami, Y., Miura, S., . . . Suzuki, K. (2013). Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exerc Immunol Rev, 19*(19), 72-85.

Lembke, P., Capodice, J., Hebert, K., & Swenson, T. (2014). Influence of omega-3 (n3) index on performance and wellbeing in young adults after heavy eccentric exercise. *J Sports Sci Med, 13*(1), 151-156.

Lenn, J., Uhl, T., Mattacola, C., Boissonneault, G., Yates, J., Ibrahim, W., & Bruckner, G. (2002a). The effects of fish oil and isoflavones on delayed onset muscle soreness. *Medicine & Science in Sports & Exercise, 34*(10), 1605-1613.

Lenn, J., Uhl, T., Mattacola, C., Boissonneault, G., Yates, J., Ibrahim, W., & Bruckner, G. (2002b). The effects of fish oil and isoflavones on delayed onset muscle soreness. *Medicine And Science In Sports And Exercise, 34*(10), 1605-1613.

Lewis, E. J., Radonic, P. W., Wolever, T. M., & Wells, G. D. (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), 28.

Li, K., Huang, T., Zheng, J., Wu, K., & Li, D. (2014). Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor α: a meta-analysis. *PloS one, 9*(2), e88103.

Markworth, J. F., Vella, L., Lingard, B. S., Tull, D. L., Rupasinghe, T. W., Sinclair, A. J., . . . Cameron-Smith, D. (2013). Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *305*(11), R1281-R1296.

Martin, S. A., Pence, B. D., & Woods, J. A. (2009). Exercise and respiratory tract viral infections. *Exercise and sport sciences reviews*, *37*(4), 157.

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P., . . . Altman, D. G. (2012). CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *International journal of surgery, 10*(1), 28-55.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2010). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *International journal of surgery, 8*(5), 336-341.

Moreira, A., Kekkonen, R. A., Delgado, L., Fonseca, J., Korpela, R., & Haahtela, T. (2007). Nutritional modulation of exercise-induced immunodepression in athletes: a systematic review and meta-analysis. *European Journal of Clinical Nutrition, 61*(4), 443-460. doi:10.1038/sj.ejcn.1602549

Nieman, D. C., Henson, D. A., McAnulty, S. R., Jin, F., & Maxwell, K. R. (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), 536-546.

Ochi, E., & Tsuchiya, Y. (2018). Eicosahexanoic Acid (EPA) and Docosahexanoic Acid (DHA) in Muscle Damage and Function. *Nutrients, 10*(5). doi:10.3390/nu10050552

Panza, V. S. P., Diefenthaeler, F., & da Silva, E. L. (2015). Benefits of dietary phytochemical supplementation on eccentric exercise-induced muscle damage: Is including antioxidants enough? *Nutrition, 31*(9), 1072-1082.

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., & Coombes, J. S. (2005). Plasma cytokine changes in relation to exercise intensity and muscle damage. *European journal of applied physiology, 95*(5-6), 514-521.

Peoples, G. E., McLennan, P. L., Howe, P. R., & Groeller, H. (2008). Fish oil reduces heart rate and oxygen consumption during exercise. *Journal of cardiovascular pharmacology, 52*(6), 540-547.

Philippou, A., Maridaki, M., Theos, A., & Koutsilieris, M. (2012). Cytokines in muscle damage. *Advances in clinical chemistry, 58*(issue), 49-97.

Poprzecki, S., Zajac, A., Chalimoniuk, M., Waskiewicz, Z., & Langfort, J. (2009). Modification of blood antioxidant status and lipid profile in response to high-intensity endurance exercise after low doses of ω-3 polyunsaturated fatty acids supplementation in healthy volunteers. *International Journal of Food Sciences & Nutrition, 60*, 67-79. doi:10.1080/09637480802406161

Saiiari, A., & Boyerahmadi, A. (2014). The Effect of Endurance Training and Omega-3 Supplementation on Inflammatory Cytokines in Endurance Runners. *International Journal of Basic Sciences and Applied Research, 3*(9), 601-605.

Santos, Levada-Pires, A. C., Alves, S. R., Pithon-Curi, T. C., Curi, R., & Cury-Boaventura, M. F. (2013). Effects of DHA-rich fish oil supplementation on lymphocyte function before and after a marathon race. *International journal of sport nutrition and exercise metabolism, 23*(2), 161-169.

Santos, E. P., Silva, A. S., Costa, M. J. d. C., Moura Junior, J. S., Quirino, E. L. d. O., Franca, G. A. M., & Asciutti, L. S. R. (2012). Omega-3 supplementation attenuates the production of C-reactive protein in military personnel during 5 days of intense physical stress and nutritional restriction. *Biology of Sport, 29*(2), 93-99.

Șerban, G., & Răzvan, G. (2017). The potential role of omega-3 fatty acids supplements in increasing athletic performance. *Timisoara Physical Education and Rehabilitation Journal, Vol 9, Iss 16, Pp 25-34 (2017)*(16), 25. doi:10.1515/tperj

Simopoulos, A. P. (2007). Omega-3 fatty acids and athletics. *Current sports medicine reports, 6*(4), 230-236.

Skarpańska-Stejnborn, A., Pilaczyńska-Szcześniak, Ł., Basta, P., Foriasz, J., & Arlet, J. (2010). Effects of supplementation with Neptune krill oil (euphasia superba) on selected redox parameters and pro-inflammatory markers in athletes during exhaustive exercise. *Journal of human kinetics, 25*, 49-57.

Slattery, K., Bentley, D., & Coutts, A. J. (2015). The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in athletes: implications of antioxidant supplementation on physiological adaptation during intensified physical training. *Sports Medicine, 45*(4), 453-471.

Suzuki, K. (2018). *Cytokine Response to Exercise and Its Modulation* (Vol. 7).

Tartibian, B., Maleki, B. H., & Abbasi, A. (2011). Omega-3 fatty acids supplementation attenuates inflammatory markers after eccentric exercise in untrained men. *Clinical Journal of Sport Medicine*, *21*(2), 131-137.

Tee, J. C., Bosch, A. N., & Lambert, M. I. (2007). Metabolic consequences of exercise-induced muscle damage. *Sports Medicine, 37*(10), 827-836.

Toft, A. D., Thorn, M., Ostrowski, K., Asp, S., Møller, K., Iversen, S., . . . Pedersen, B. K. (2000). N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. *Journal of applied physiology, 89*(6), 2401-2406.

CHAPTER 4: GENERAL DISCUSSION AND CONCLUSION

4.1 Introduction

Large volumes of unaccustomed, intense exercise in recreational and competitive athletes cause increased muscle damage, inflammation and suppression of the immune system resulting in delayed exercise recovery, overtraining syndrome, and compromised exercise performance. Available evidence suggests that omega-3 (n-3) polyunsaturated fatty acids (PUFAs) may alter the exercise-induced inflammatory response and have immunomodulatory effects in recreational and competitive athletes, however the evidence regarding this topic is contradicting. Therefore, the specific objectives were to systematically review the effect of n-3 PUFA supplementation compared to a placebo/control on:

- The markers of inflammation including cytokines (TNF-α, IL-2, -4, -6 and 10) and acute-phase proteins (CRP) in recreational and competitive athletes.
- Muscle damage marker (creatine kinase) in recreational and competitive athletes and;
- Exercise performance measured through time trials and time to exhaustion in recreational and competitive athletes

4.2 Summary of objectives

4.2.1 Objective 1: Effect of n-3 PUFA supplementation on markers of inflammation including cytokines (TNF-α, IL-2, -4, -6 and 10) and acute-phase proteins (CRP) in recreational and competitive athletes.

The first objective was addressed through the process of data extraction from studies conducted on recreational and competitive athletes predominantly receiving n-3 PUFA supplementation. Following the initial search and selecting the studies that met the set inclusion criteria, 11 studies were included for the systematic review and meta-analysis.

A suggestive trend towards a significant beneficial effect for n-3 PUFA supplementation to increase anti-inflammatory marker IL-10 and decrease pro-inflammatory marker CRP was demonstrated. Cytokine IL-10 is produced upon stimulation by IL-6 and is known to have potent anti-inflammatory effects through inhibiting the production of potent pro-inflammatory cytokines IL-1β, IL-6 & TNF-α as well as ROS. This possible increase of IL-10 could restrict the magnitude and duration of exercise-induced inflammatory response, therefore improving post-exercise recovery. The possible decrease in CRP suggests that n-3 PUFA has immunomodulatory effects

in lowering exercise-induced inflammation. Although the current meta-analysis indicates a suggestive beneficial effect of n-3 PUFA supplementation a clear conclusion could not be drawn from the results due to the low quality of the studies as well as the small sample of studies included. Therefore, high quality future research is needed to come to a clear conclusion on the effect of n-3 PUFA supplementation on recreational and competitive athletes. Since there is no evidence to whether IL-10 and CRP is specific to the intensity, duration or volume of an exercise protocol, supplementation of n-3 PUFAs could be recommended to all kind of athletes (recreational or competitive athletes). Furthermore, no significant effect of n-3 PUFA supplementation was found for the remaining markers TNF-α, IL-2, IL-4 and IL-6.

4.2.2 Objective 2: Effect of n-3 PUFA supplementation on markers of muscle damage

The second objective was determining the effect of n-3 PUFA supplementation on muscle damage marker CK. Seven studies were included for the analysis of muscle damage marker CK, no significant effects of n-3 PUFA supplementation was found for the muscle damage marker. Creatine kinase is a compact enzyme used as an indirect marker of muscle damage found in the cytosol and mitochondria of high energy demand tissues (e.g. skeletal muscles) that forms the core of the phosphocreatine (PCr) circuit (Baird *et al.*, 2012). Moreover, CK is important for the regeneration of cellular ATP through catalysing the reversible phosphorylation of creatine to phosphocreatine and of ADP to ATP (Baird *et al.*, 2012). Therefore, CK is partly dependent on the type of exercise (i.e. aerobic and anaerobic exercise). Although no beneficial effect of n-3 PUFA supplementation was found post-exercise, the intake of this supplementation can still be beneficial for improving recovery as indicated by individual studies included in this systematic review and meta-analysis did show a decrease in CK concentrations in resistance trained individuals. Since the side effects of n-3 PUFA supplementation reported are only experienced when taking >4 g/day this supplementation can be recommended to recreational and competitive athletes taking part in resistance training. The recommended daily dosage for healthy individuals is > 2 g/day since some studies failed to show any cytokine production on lower dosages (Calder, 2017). Currently there are no recommendations regarding n-3 PUFA intake specifically for recreational and competitive athletes, therefore 2 – 4 g/day can be recommended.

4.2.3 Objective 3: Effect of n-3 PUFA supplementation on exercise performance

Limited studies were available for the assessment of exercise performance in terms of time to exhaustion and time to complete a specific distance (time trials). Five studies were included to determine the effect of n-3 PUFA supplementation on exercise performance. Omega-3 PUFA

supplementation did not show any significant effects on either time to exhaustion or time trial performance. Exercise performance mostly dependent on muscle strength, endurance and cardiovascular capacity and since n-3 PUFA supplementation only demonstrated some effects on cardiovascular capacity the potential of n-3 PUFA supplementation to improve exercise performance is unlikely. Therefore, supplementation with n-3 PUFAs can be recommended to recreational and competitive athletes to improve cardiovascular capacity but more studies is needed to review the overall effect of supplementation on exercise performance.

4.3 Risk of bias and quality assessment

The risk of bias in all included studies were determined by using the Cochrane Collaboration tool (Higgins *et al.*, 2011). Studies were assessed with regard to random sequence generation, allocation concealment, blinding, outcome assessment, incomplete outcome data, selective reporting and other sources of bias. The overall risk of bias in terms of random sequence generation and allocation concealment was high as the method of randomisation and blinding was not well described or reported in all the studies. Performance bias was also found to be one of the more prevalent types of bias. Additional sources of bias were difficult to assess as reporting on various aspects within the trials was poor. Future studies should aim to address all sources of bias appropriately.

The CONSORT checklist was used to evaluate the quality of reporting of the included studies. The quality of the studies was deemed low since the methodological factors such as power calculations, randomisations, blinding of participants and researchers, compliance of supplementation intake and the reporting of data was mostly not reported. Future studies should aim to address the reporting of important methodologic factors.

4.4 Strengths and limitations of this review

The present work has some remarkable strength points. Firstly, it is based on a robust methodology applied to current and comprehensive evidence regarding n-3 PUFA supplementation on athletes and active individuals. In addition, an up to date and critical evaluation about the most common conditions under which the effect of n-3 PUFA supplementation is evaluated using RCT was provided. Naturally, the present work had certain weak points as well. For example, a high degree of methodological heterogeneity among studies was reported, the included studies were based on small sample sizes with limited duration and most of the studies did not provide power calculation to justify the sample size considered. On the other hand, these limiting factors provide useful suggestions to scientists involved in

randomised controlled studies aimed at assessing the beneficial effects of n-3 PUFA supplementation on athletes and active individuals.

The lack of studies (small number of studies) and available data was the main limitation of this systematic review and meta-analysis. In order to perform a meta-analysis, original data is required in a specific format that is not always reported in the included published articles (e.g. means and standard deviations). Although the respective authors were emailed at least two times, the response rate was very poor and we managed to get feedback from only two of the six authors.

Not all studies reported on the same outcomes or used the same measuring techniques for the inflammatory markers. For example, high sensitivity ELISA kits and commercially available ELISA kit were used to measure the inflammatory markers, since these kits are measured at different wave lengths and the reference ranges differ enormously, they are thus not comparable. A high degree of methodological heterogeneity among studies was reported, the included studies were based on small sample sizes due to the specific population group (athletes and active individuals) with limited duration and most of the studies did not provide power calculation to justify the sample size considered.

It's also important to note that systematic reviews are also subject to publication bias as some trials might have been missed or trials with negative results might not necessarily have been published. However, all efforts have been made to ensure that as many databases as possible were screened.

4.5 Conclusion and recommendations

Due to the small number of studies included in the current systematic review and meta-analysis, we failed to come to a clear conclusion regarding the effect of n-3 PUFA supplementation on exercise performance. This systematic review and meta-analysis did demonstrate a possible effect of n-3 PUFA supplementation on attenuating the pro-inflammatory response, particularly CRP, and improving the anti-inflammatory response, particularly IL-10. Moreover, the present study also demonstrates the need for more reliable evidence in order to provide sufficient recommendation to recreational and competitive athletes regarding the effect of n-3 PUFA supplementation.

A few recommendations can also be made regarding the planning of new RCTs to evaluate the effect of n-3 PUFA supplementation on inflammation, muscle damage and exercise performance. Firstly, it is important for the RCT to have a significant statistical power and large enough sample size. Secondly, the target population of athletes should be specified along with the specific evaluation of the training schedule and the dose and form of n-3 PUFA supplementation along

with the duration of the RCT should be adequate. Thirdly, the techniques used for biomarker assessment should be standardized. The above-mentioned recommendations should be taken into account in the phase of study planning to provide robust and useful evidence.

REFERENCES

Andrade, P.M., Ribeiro, B.G., Bozza, M.T., Rosa, L.F.B.C. & do Carmo, M.G.T. 2007. Effects of the fish-oil supplementation on the immune and inflammatory responses in elite swimmers. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 77(3):139-145.

Ariel, A. & Serhan, C.N. 2007. Resolvins and protectins in the termination program of acute inflammation. *Trends Immunology*, 28(4):176-183.

Atashak', S., Sharafi', H., Azarbayjani, M.A., Stannard, S.R., Goli', M.A. & Haghighi', M.M. 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. *Kinisiology*, 45(1):22-29.

Atashak, S., Sharafi, H., Azarbayjani, M.A., Goli, M.A., Batoorak, K. & Karimi, W. 2012. Effect of omega-3 fatty acids on lipid peroxidation and plasma total antioxidant capacity after acute resistance exercise in young male athletes. *Scientific Journal of Kurdistan University of Medical Sciences*, 17(3):Pe51-Pe59, En57.

Baird, M.F., Graham, S.M., Baker, J.S. & Bickerstaff, G.F. 2012. Creatine-kinase-and exercise-related muscle damage implications for muscle performance and recovery. *Journal of nutrition and metabolism*, 2012.

Baumert, P., Lake, M.J., Stewart, C.E., Drust, B. & Erskine, R.M. 2016. Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing. *European Journal of Applied Physiology*, 116(9):1595-1625.

Bloomer, R.J., Larson, D.E., Fisher-Wellman, K.H., Galpin, A.J. & Schilling, B.K. 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 Health Dis*, 8:36.

Brancaccio, P., Lippi, G. & Maffulli, N. 2010. Biochemical markers of muscular damage. *Clinical Chemistry and Laboratory Medicine*, 48(6):757-767.

Brentano, M. & Martins Kruel, L. 2011. A review on strength exercise-induced muscle damage: applications, adaptation mechanisms and limitations. *J Sports Med Phys Fitness*, 51(1):1-10.

Bresciani, G., da Cruz, I.B.M. & González-Gallego, J. 2015. Chapter four-manganese superoxide dismutase and oxidative stress modulation. *Advances in clinical chemistry*, 68:87-130.

Browning, L.M., Walker, C.G., Mander, A.P., West, A.L., Madden, J., Gambell, J.M., Young, S., Wang, L., Jebb, S.A. & Calder, P.C. 2012. Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish–. *The American journal of clinical nutrition*, 96(4):748-758.

Buckley, C.D., Gilroy, D.W. & Serhan, C.N. 2014. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity*, 40(3):315-327.

Buckley, J.D., Burgess, S., Murphy, K.J. & Howe, P.R. 2009. DHA-rich fish oil lowers heart rate during submaximal exercise in elite Australian Rules footballers. *Journal of Science and Medicine in Sport*, 12(4):503-507.

Calder, P.C. 2006. Polyunsaturated fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 75(3):197-202.

Calder, P.C. 2008. The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 79(3-5):101-108.

Calder, P.C. 2010. Omega-3 fatty acids and inflammatory processes. *Nutrients*, 2(3):355-374.

Calder, P.C. 2015. Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1851(4):469-484.

Calder, P.C. 2017. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochemical Society Transactions*, 45(5):1105-1115.

Capó, X., Martorell, M., Llompart, I., Sureda, A., Tur, J.A. & Pons, A. 2014. Docosahexanoic acid diet supplementation attenuates the peripheral mononuclear cell inflammatory response to exercise following LPS activation. *Cytokine*, 69:155-164.

Carbal-Santos, C., Gerosa-Neto, J., Inoue, D.S., Panissa, V.L.G., Gobbo, L.A., Zagatto, A.M., Campos, E.Z. & Lira, S.L. 2015. Similar anti-inflammatory acute reponses from moderate-intensity continuous and high-intensity intermittent exercise. *Journal of sport science and medicine*, 14:849-856.

Collins, Y., Chouchani, E.T., James, A.M., Menger, K.E., Cochemé, H.M. & Murphy, M.P. 2012. Mitochondrial redox signalling at a glance. *J Cell Sci*, 125(4):801-806.

Da Boit, M., Hunter, A.M. & Gray, S.R. 2017. Fit with good fat? The role of n-3 polyunsaturated fatty acids on exercise performance. *Metabolism*, 66:45-54.

Da Boit, M., Mastalurova, I., Brazaite, G., McGovern, N., Thompson, K. & Gray, S.R. 2015. The effect of krill oil supplementation on exercise performance and markers of immune function. *PloS one*, 10(9):e0139174.

Dalli, J., Colas, R.A. & Serhan, C.N. 2013. Novel n-3 immunoresolvents: structures and actions. *Sci Rep*, 3:1940.

Delfan, M., Ebrahim, K., Baesi, F., Mirakhori, Z., Ghalamfarsa, G., Bakhshaei, P., Saboor-Yaraghi, A.A., Razavi, A., Setayesh, M. & Yousefi, M. 2015. The immunomodulatory effects of fish-oil supplementation in elite paddlers: A pilot randomized double blind placebo-controlled trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 99:35-40.

Fisher-Wellman, K. & Bloomer, R.J. 2009. Acute exercise and oxidative stress: a 30 year history. *Dynamic Medicine*, 8(1):1.

Forbes, S.J. & Rosenthal, N. 2014. Preparing the ground for tissue regeneration: from mechanism to therapy. *Nature medicine*, 20(8):857-869.

Fredman, G. & Serhan, C.N. 2011. Specialized proresolving mediator targets for RvE1 and RvD1 in peripheral blood and mechanisms of resolution. *Biochem J*, 437(2):185-197.

Fullerton, J.N., O'Brien, A.J. & Gilroy, D.W. 2014. Lipid mediators in immune dysfunction after severe inflammation. *Trends Immunol*, 35(1):12-21.

Gilroy, D. & De Maeyer, R. 2015. New insights into the resolution of inflammation. (*In*. Seminars in immunology organised by: Elsevier. p. 161-168).

Gleeson, M. 2007. Immune function in sport and exercise. *J Appl Physiol (1985)*, 103(2):693-699.

Gray, P., Chappell, A., Jenkinson, A.M., Thies, F. & Gray, S.R. 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):206-214.

Gray, P., Gabriel, B., Thies, F. & Gray, S.R. 2012. Fish oil supplementation augments post-exercise immune function in young males. *Brain Behavior and Immunity*, 26:1265-1272.

Gruys, E., Toussaint, M.J.M., Niewold, T.A. & Koopmans, S.J. 2005. Acute phase reaction and acute phase proteins. *Journal of Zhejiang University. Science. B*, 6(11):1045-1056.

Hackney, A.C. & Koltun, K.J. 2012. The immune system and overtraining in athletes: clinical implications. *Acta Clin Croat*, 51(4):633-641.

Hensley, K., Robinson, K.A., Prasad, G., Salsman, S. & Floyd, R.A. 2000. Reactive oxygen species, cell signaling, and cell injury. *Free radical biology & medicine*, 28(10):1456-1462.

Hingley, L., Macartney, M.J., Brown, M.A., McLennan, P.L. & Peoples, G.E. 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*, 27(4):335-343.

Hosseini, A., Peeri, M., Azarbayjani, M.A. & Mateenhomaei, H. 2014. EFFECT OF RESISTANCE TRAINING AND OMEGA3 SUPPLEMENTATION ON MARKERS OF MUSCLE DAMAGE AND INFLAMMATION IN TRAINED MEN.

Hosseini, A., Peeri, M., Azarbayjani, M.A. & Mateenhomaei, H. 2015. Effect of resistance training and omega3 supplementation on markers of muscle damage and inflammation in trained men. *Indian Journal of Fundamental and Applied Life Sciences*, 5(2):122-132.

Howatson, G. & Van Someren, K.A. 2008. The prevention and treatment of exercise-induced muscle damage. *Sports medicine*, 38(6):483-503.

Huffman, D.M., Altena, T.S., Mawhinney, T.P. & Thomas, T.R. 2004. Effect of n-3 fatty acids on free tryptophan and exercise fatigue. *European journal of applied physiology*, 92(4-5):584-591.

Hyldahl, R.D., Chen, T.C. & Nosaka, K. 2017. Mechanisms and mediators of the skeletal muscle repeated bout effect. *Exercise and sport sciences reviews*, 45(1):24-33.

Hyldahl, R.D. & Hubal, M.J. 2014. Lengthening our perspective: morphological, cellular, and molecular responses to eccentric exercise. *Muscle & nerve*, 49(2):155-170.

Jackson, M.J. 2015. Redox regulation of muscle adaptations to contractile activity and aging. *Journal of Applied Physiology*, 119(3):163-171.

Jackson, M.J., Pye, D. & Palomero, J. 2007. The production of reactive oxygen and nitrogen species by skeletal muscle. *Journal of Applied Physiology*, 102(4):1664-1670.

Jakeman, J., Lambrick, D., Wooley, B., Babraj, J., Faulkner, J., Jakeman, J.R., Lambrick, D.M., Babraj, J.A. & Faulkner, J.A. 2017. Effect of an acute dose of omega-3 fish oil following exercise-induced muscle damage. *European Journal of Applied Physiology*, 117(3):575-582.

James, M.J., Gibson, R.A. & Cleland, L.G. 2000. Dietary polyunsaturated fatty acids and inflammatory mediator production. *The American journal of clinical nutrition*, 71(1):343s-348s.

Ji, L.L., Kang, C. & Zhang, Y. 2016. Exercise-induced hormesis and skeletal muscle health. *Free Radical Biology and Medicine*.

Kanda, K., Sugama, K., Hayashida, H., Sakuma, J., Kawakami, Y., Miura, S., Yoshioka, H., Mori, Y. & Suzuki, K. 2013. Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exerc Immunol Rev*, 19(19):72-85.

Kasapis, C. & Thompson, P.D. 2005. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *Journal of the american College of Cardiology*, 45(10):1563-1569.

Kawabata, F., Neya, M., Hamazaki, K., Watanabe, Y., Kobayashi, S. & Tsuji, T. 2014. Supplementation with eicosapentaenoic acid-rich fish oil improves exercise economy and reduces perceived exertion during submaximal steady-state exercise in normal healthy untrained men. *Bioscience, biotechnology, and biochemistry*, 78(12):2081-2088.

Kendall, B. & Eston, R. 2002. Exercise-induced muscle damage and the potential protective role of estrogen. *Sports medicine*, 32(2):103-123.

Kim, J. & Lee, J. 2014. A review of nutritional intervention on delayed onset muscle soreness. Part I. *J Exerc Rehabil*, 10(6):349-356.

Koch, A.J. 2010. Immune response to resistance exercise. *American Journal of Lifestyle Medicine*, 4(3):244-252.

Kohli, P. & Levy, B.D. 2009. Resolvins and protectins: mediating solutions to inflammation. *British journal of pharmacology*, 158(4):960-971.

Lembke, P., Capodice, J., Hebert, K. & Swenson, T. 2014. Influence of omega-3 (n3) index on performance and wellbeing in young adults after heavy eccentric exercise. *J Sports Sci Med*, 13(1):151-156.

Lenn, J., Uhl, T., Mattacola, C., Boissonneault, G., Yates, J., Ibrahim, W. & Bruckner, G. 2002. The effects of fish oil and isoflavones on delayed onset muscle soreness. *Medicine & Science in Sports & Exercise*, 34(10):1605-1613.

Lewis, E.J., Radonic, P.W., Wolever, T.M. & Wells, G.D. 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):28.

Macartney, M.J., Hingley, L., Brown, M.A., Peoples, G.E. & McLennan, P.L. 2014. Intrinsic heart rate recovery after dynamic exercise is improved with an increased omega-3 index in healthy males. *British Journal of Nutrition*, 112(12):1984-1992.

Markworth, J.F., Vella, L., Lingard, B.S., Tull, D.L., Rupasinghe, T.W., Sinclair, A.J., Maddipati, K.R. & Cameron-Smith, D. 2013. Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 305(11):R1281-R1296.

Meeusen, R., Duclos, M., Foster, C., Fry, A., Gleeson, M., Nieman, D., Raglin, J., Rietjens, G., Steinacker, J. & Urhausen, A. 2013. Prevention, diagnosis, and treatment of the overtraining syndrome: joint consensus statement of the European College of Sport Science and the American College of Sports Medicine. *Medicine and science in sports and exercise*, 45(1):186-205.

Moldoveanu, A.I., Shephard, R.J. & Shek, P.N. 2001. The cytokine response to physical activity and training. *Sports Medicine*, 31(2):115-144.

Mori, T.A. & Beilin, L.J. 2004. Omega-3 fatty acids and inflammation. *Current atherosclerosis reports*, 6(6):461-467.

Nieman, D.C., Henson, D.A., McAnulty, S.R., Jin, F. & Maxwell, K.R. 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):536-546.

Niess, A.M. & Simon, P. 2007. Response and adaptation of skeletal muscle to exercise–the role of reactive oxygen species. *Front Biosci*, 12(12):4826-4838.

Oostenbrug, G., Mensink, R., Hardeman, M., De Vries, T., Brouns, F. & Hornstra, G. 1997. Exercise performance, red blood cell deformability, and lipid peroxidation: effects of fish oil and vitamin E. *Journal of applied physiology*, 83(3):746-752.

Ortega, E. 2008. Exercise and the immune system. *Proceedings of the Nutrition Society*, 67(OCE1):E5.

Panza, V.S.P., Diefenthaeler, F. & da Silva, E.L. 2015. Benefits of dietary phytochemical supplementation on eccentric exercise-induced muscle damage: Is including antioxidants enough? *Nutrition*, 31(9):1072-1082.

Paulsen, G., Mikkelsen, U.R., Raastad, T. & Peake, J.M. 2012. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise. *Exerc Immunol Rev*, 18(1):42-97.

Peake, J.M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K. & Coombes, J.S. 2005. Plasma cytokine changes in relation to exercise intensity and muscle damage. *European journal of applied physiology*, 95(5-6):514-521.

Peoples, G.E., McLennan, P.L., Howe, P.R. & Groeller, H. 2008. Fish oil reduces heart rate and oxygen consumption during exercise. *Journal of cardiovascular pharmacology*, 52(6):540-547.

Petersen, A.M.W. & Pedersen, B.K. 2005. The anti-inflammatory effect of exercise. *Journal of applied physiology*, 98(4):1154-1162.

Philippou, A., Maridaki, M., Theos, A. & Koutsilieris, M. 2012. Cytokines in muscle damage. *Advances in clinical chemistry*, 58(issue):49-97.

Pillon, N.J., Bilan, P.J., Fink, L.N. & Klip, A. 2013. Cross-talk between skeletal muscle and immune cells: muscle-derived mediators and metabolic implications. *American Journal of Physiology-Endocrinology and Metabolism*, 304(5):E453-E465.

Pingitore, A., Lima, G.P., Mastorci, F., Quinones, A., Iervasi, G. & Vassalle, C. 2015. Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. *Nutrition*, 31(7-8):916-922.

Poprzecki, S., Zajac, A., Chalimoniuk, M., Waskiewicz, Z. & Langfort, J. 2009. Modification of blood antioxidant status and lipid profile in response to high-intensity endurance exercise after low doses of ω-3 polyunsaturated fatty acids supplementation in healthy volunteers. *International Journal of Food Sciences & Nutrition*, 60:67-79.

Powers, S.K. & Jackson, M.J. 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological reviews*, 88(4):1243-1276.

Proske, U. & Morgan, D. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of physiology*, 537(2):333-345.

Radoman, K., Vucic, V., Arsic, A., Cubrilo, D., Jeremic, N., Jeremic, J. & Jakovljevic, V. 2015. Effects of different PUFA supplementation on inflammatory response markers in young soccer players. *Serbian Journal of Experimental and Clinical Research*, 16(4):305-311.

Ránky, M., Köteles, F., Nyakas, C., Bárdos, G. & Szabo, A. 2017. A 90 DAY SUPPLEMENTATION OF POLYUNSATURATED FATTY ACIDS (PUFA) HAS BENEFITS ON HEALTH MEASURES AND EXERCISE PERFORMANCE. *Baltic Journal of Sport & Health Sciences*(1):36-43.

Raphael, W. & Sordillo, L.M. 2013. Dietary polyunsaturated fatty acids and inflammation: the role of phospholipid biosynthesis. *International journal of molecular sciences*, 14(10):21167-21188.

Rees, D., Miles, E.A., Banerjee, T., Wells, S.J., Roynette, C.E., Wahle, K.W. & Calder, P.C. 2006. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men–. *The American journal of clinical nutrition*, 83(2):331-342.

Rowlands, D.S., Pearce, E., Aboud, A., Gillen, J., Gibala, M., Donato, S., Waddington, J., Green, J. & Tarnopolsky, M. 2012. Oxidative stress, inflammation, and muscle soreness in an 894-km relay trail run. *European journal of applied physiology*, 112(5):1839-1848.

Saiiari, A. & Boyerahmadi, A. 2014. The Effect of Endurance Training and Omega-3 Supplementation on Inflammatory Cytokines in Endurance Runners. *International Journal of Basic Sciences and Applied Research*, 3(9):601-605.

Santos, Levada-Pires, A.C., Alves, S.R., Pithon-Curi, T.C., Curi, R. & Cury-Boaventura, M.F. 2013. Effects of DHA-rich fish oil supplementation on lymphocyte function before and after a marathon race. *International journal of sport nutrition and exercise metabolism*, 23(2):161-169.

Santos, E.P., Silva, A.S., Costa, M.J.d.C., Moura Junior, J.S., Quirino, E.L.d.O., Franca, G.A.M. & Asciutti, L.S.R. 2012. Omega-3 supplementation attenuates the production of C-reactive protein in military personnel during 5 days of intense physical stress and nutritional restriction. *Biology of Sport*, 29(2):93-99.

Serhan, C.N., Chiang, N. & Dalli, J. 2015a. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. *Semin Immunol*, 27(3):200-215.

Serhan, C.N., Chiang, N., Dalli, J. & Levy, B.D. 2015b. Lipid mediators in the resolution of inflammation. *Cold Spring Harb Perspect Biol*, 7(2):a016311.

Serhan, C.N. & Petasis, N.A. 2011. Resolvins and protectins in inflammation resolution. *Chem Rev*, 111(10):5922-5943.

Serhan, C.N. & Recchiuti, A. 2012. Pro-resolving lipid mediators (SPMs) and their actions in regulating miRNA in novel resolution circuits in inflammation. *Frontiers in immunology*, 3:298.

Silva, F.O.C.d. & Macedo, D.V. 2011. Physical exercise, inflammatory process and adaptive condition: an overview. *Revista Brasileira de Cineantropometria & Desempenho Humano*, 13(4):320-328.

Skarpańska-Stejnborn, A., Pilaczyńska-Szcześniak, Ł., Basta, P., Foriasz, J. & Arlet, J. 2010. Effects of supplementation with Neptune krill oil (euphasia superba) on selected redox parameters and pro-inflammatory markers in athletes during exhaustive exercise. *Journal of human kinetics*, 25:49-57.

Slattery, K., Bentley, D. & Coutts, A.J. 2015. The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in athletes: implications of antioxidant supplementation on physiological adaptation during intensified physical training. *Sports Medicine*, 45(4):453-471.

Steinbacher, P. & Eckl, P. 2015. Impact of oxidative stress on exercising skeletal muscle. *Biomolecules*, 5(2):356-377.

Sterz, K., Scherer, C. & Ecker, J. 2015. A simple and robust UPLC-SRM/MS method to quantify urinary eicosanoids. *Journal of lipid research*:1-26.

Suzuki, K. 2018. Cytokine Response to Exercise and Its Modulation. Vol. 7.

Tee, J.C., Bosch, A.N. & Lambert, M.I. 2007. Metabolic consequences of exercise-induced muscle damage. *Sports Medicine*, 37(10):827-836.

Terra, R., Silva, S.A.G.d., Pinto, V.S. & Dutra, P.M.L. 2012. Effect of exercise on immune system: response, adaptation and cell signaling. *Revista Brasileira de Medicina do Esporte*, 18(3):208-214.

Tidball, J.G. 2005. Inflammatory processes in muscle injury and repair. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 288(2):R345-R353.

Tidball, J.G. 2011. Mechanisms of muscle injury, repair, and regeneration. *Compr Physiol*, 1(4):2029-2062.

Toft, A.D., Thorn, M., Ostrowski, K., Asp, S., Møller, K., Iversen, S., Hermann, C., Søndergaard, S.R. & Pedersen, B.K. 2000. N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. *Journal of applied physiology*, 89(6):2401-2406.

Urso, M.L. & Clarkson, P.M. 2003. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, 189(1):41-54.

Wood, K., Lau, A., Mantzioris, E., Gibson, R., Ramsden, C. & Muhlhausler, B. 2014. A low omega-6 polyunsaturated fatty acid (n-6 PUFA) diet increases omega-3 (n-3) long chain PUFA status in plasma phospholipids in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 90(4):133-138.

Yavari, A., Javadi, M., Mirmiran, P. & Bahadoran, Z. 2015. Exercise-induced oxidative stress and dietary antioxidants. *Asian journal of sports medicine*, 6(1).

Żebrowska, A., Mizia-Stec, K., Mizia, M., Gąsior, Z. & Poprzęcki, S. 2015. Omega-3 fatty acids supplementation improves endothelial function and maximal oxygen uptake in endurance-trained athletes. *European journal of sport science*, 15(4):305-314.

ADDENDUM A: CONSORT CHECKLIST

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	

Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

ADDENDUM B: EXAMPLE OF DATA EXTRACTION FORM AND RISK OF BIAS

Author (year)		
Methods	Study design: Study duration:	
Participants	Population: Total number randomised: Country and setting: Inclusion criteria: Exclusion criteria:	
Intervention	Experimental supplementation: Placebo supplementation:	
Outcomes	Inflammatory markers: Muscle damage marker: Exercise performance measurement:	
Notes	Exercise protocol: Ethics approval: Financial contributors:	
Risk of bias		
Bias	Authors' judgement	Support of judgement
Random sequence generation (selection bias)	High risk Unclear risk Low risk	
Allocation concealment (selection bias)	High risk Unclear risk Low risk	
Blinding of participants and personnel (performance bias)	High risk Unclear risk Low risk	

Blinding of outcome assessment (detection bias)	High risk Unclear risk Low risk	
Incomplete outcome data (attrition bias)	High risk Unclear risk Low risk	
Selective reporting (reporting bias)	High risk Unclear risk Low risk	
Other bias	High risk Unclear risk Low risk	