Effectiveness of omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation on endurance capacity, performance, and neuromuscular fatigue in cyclists.

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Abstract

Cycling performance requires the optimisation of physiological, psychological, biomechanical and aerodynamic factors. Research pertaining to success in road cycling indicates that physiological variables such as maximal oxygen uptake and lactate threshold are key performance indicators. Additionally, sustaining high power output over prolonged periods can lead to exercise-induced fatigue and muscle damage. To mitigate these effects, omega-3 fatty acids, known for their anti-inflammatory properties, have been proposed as a potential ergogenic aid, with some evidence suggesting it may reduce muscle damage and perceived muscle soreness in athletes. The aim of this thesis was to quantify the effectiveness of omega-3 supplementation on endurance performance, recovery and neuromuscular fatigue (NMF) in cyclists. Initially a systematic review was conducted (*n* = 11), which identified that ~1.05 $g \cdot day^{-1}$ was the lowest dose to begin demonstrating positive results within endurance performances and during the recovery post performances. However, due to the limited number of studies that utilised NMF tests or endurance protocols, no definitive conclusions can be made regarding the effectiveness of omega-3.

From these findings, we sought to investigate whether a high dosage (1600 mg) of omega-3 ingested daily for 8-weeks within a commercially available beverage, could enhance endurance performance and reduce NMF in well-trained cyclists. Six non-professional road cyclists and triathletes (age 45.7 [34.8] years; height 182.5 [176.7] cm; body mass 80.0 [4.5] kg) participated in the study, attending the laboratory on four separate occasions over a 10-week period. The first two visits included: (1) an 8-site skinfold assessment and a maximal oxygen uptake (VO_2max) test on a cycle ergometer (week 1 [visit 1]), and (2) a 75-minute steady-state cycle (SSC) proceeded by a 16.1 km time-trial (TT) in week 2 (visit 2). During visit 2, NMF was assessed using a

countermovement jump (CMJ) protocol at baseline (after SSC warm-up), post-SSC, and post-TT. Following visit 2, participants consumed the commercially available omega-3 beverage, daily for 8-weeks. Visit 3 was a repeat of visit 2 at week 9, followed by visit 4 which was a repeat of visit 1 in week 10. Throughout the supplementation period, a food diary, training log, and athlete well-being questionnaire were completed.

No significant differences were found from pre- to post- omega-3 supplementation in VO_2max measures, mean sum of 8 skinfolds, SSC performances, TT performances, or CMJ ($p > 0.05$). Dietary analysis identified that participants failed to consume enough carbohydrate and fat intakes to meet their performance demands, based on widely accepted sport nutrition guidelines. However, omega-3 supplementation significant reduced perceived fatigue ($p < 0.001$), stress levels ($p = 0.05$), and mood ($p = 0.04$). These findings suggest that omega-3 supplementation has no significant effect on endurance capacity, performance, or NMF in well-trained athletes. However, given the limited sample size in this study coupled with the available literature on omega-3 supplementation in endurance sports, further research, with a larger sample size ($n = 44$, based on Post hoc Power calculation) is required for more direct comparisons.

Contents

List of Figures

List of Tables

Abbreviations

ALA	Alpha-linolenic Acid
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
AU	Arbitrary Units
BC	Body Composition
BMI	Body Mass Index (m^2)
BMX	Bicycle Motocross
Ca^2+	Calcium
CI	Confidence Interval
CK	Creatine Kinase
cm	centre meters
C-RP	C-reactive Protein
CMJ	Countermovement Jump
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
EFSA	European Food Safety Authorities
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAO	Food and Agriculture Organisation of the United Nations

FO	Fish Oil
g	Grams
HR	Heart Rate (b·min-1)
HPa	HectoPascals
HRmax	Maximal Hear Rate (b·min-1)
IGF-1	Insulin-like Growth factor 1
IL-1b	Interleukin 1b
IL-6	Interleukin 6
K^+	Potassium
kg	Kilograms
KJ	Kilojoules
km	Kilometeres
M/S	Meter per second
ml	Millileters
mm	Milimeters
MS	Milliseconds
N	Peak Force
n-3 PUFA	Omega-3
Na^+	Sodium
NMF	Neuromuscular Fatigue
O_2	Oxygen

Omega-3	Omega-3 fatty Acids
ORAC	Oxygen Radical Absorbtion Capacity
PCr	Phosphocreatine
PEO	Population Exposure Outcome
PIS	Participant Information Sheet
PPE	Personal Protective Equipment
PRISMA	Preferred Reporting Items for Systematic reviews and meta-Analyses
PUFA	Polyunsaturated Fatty Acids
RER	Respiratory Exchange Ratio
ROS	Reactive Oxygen Species
RPE	Rate of perceived exertion
RPM	Revolutions per minute
S	Seconds (s)
SSC	Steady State Cycle
SWEET	Square Wave Endurance Test
TT	Time Trial
V	Voltage
VAS	Visual Analog Scale
VO_2	Volume of Oxygen
VO_2max	Volume of Maximal Oxygen Consumption ($ml.kg^{-1}.min^{-1}$)

VT_2	Ventilatory Threshold
W	Watts
Wmax	Maximal Power (W)

Chapter 1. Introduction

1.1 Cycling Overview

Cycling was first introduced as a sport in the United Kingdom (UK) in the 1860's (Bigland-Ritchie, 1984). However, it was not until the 1990s that cycling was considered as the fourth most popular sport, with British Cycling (the National Governing Body of British cycling) estimating ~14,000 members in 1996. Further developments of cycling culture have led to further increases in popularity, which can partly be attributed to Sir Bradley Wiggins being the first British cyclist to win the Tour de France in 2012 (Grous, 2012). Consequently, 13,000 people joined a cycling club with a further 250 people a day joining due the success of the London Olympic Games (Grous, 2012). In 2019, British Cycling claimed that a total of 150,000 members joined a cycling club which is a three-fold increase since 2012. However, as of 2024, British Cycling have reported a total of 130,000 members, which is a decrease from 2019. However, this decrease was likely affected by the COVID-19 pandemic which restricted the ability to a part of a cycling club (Redmond et al., 2023).

Cyclists can participate in numerous sporting disciplines, such as off-road, road, track cycling, and bicycle motocross (BMX). Off-road cycling (e.g. mountain biking) is commonly performed on rough terrains and involves navigating a physically challenging courses (Impellizzeri et al., 2002; Impellizzeri & Marcora, 2007). This form of cycling tests endurance, strength, and balance on uneven terrains (Smekal et al., 2015). Road cycling is considered the most popular form of cycling discipline and is endurance focused (Lucía et al., 2001). This discipline has multiple events that cyclists can participate in, including stage-racing, one-day classics, and time trials (Lucía et al., 2001; Mignot, 2015; Vogt et al., 2006). An example of a

road cycling event is the Tour de France where athletes cycle ~3,500 km over a three-week period utilising their stamina and strategic racing abilities (Campos, 2003; Lucia et al., 2003). Track cycling involves both sprint and endurance events and is performed on specifically designed track or velodrome, which features a steep banking (Craig & Norton, 2001). Some track cycling events include the 200 m flying sprint and the Madison (Jeukendrup et al., 2000). However, BMX cycling is one of the most recent forms of cycling events (introduced in the 1970's) that requires athletes to utilise their physical skills (such as speed, strength, and agility) in a highly tactical events, for example in freestyle BMX (Mateo-March et al., 2012; Nelson, 2010; Novak & Dascombe, 2014).

One of the biggest cycling events encompassing off-road, road, track and BMX events is the Olympics Games (Grous, 2012). Cycling was first introduced in the 1896 Athens Olympic Games, with six events: road race, 10 km, 100 km, 1/3 km time trial (TT), 12 h race, and 2 km sprint (Mallon & Widlund, 2015). As of the latest Olympic Games held in Paris 2024, there are 22 cycling events which include four different disciplines including track (since 1906; Lennartz 2002), road (since 1896; Mallon & Widlund, 2015), off-road (since 1996; Savre et al., 2009), and BMX cycling (since 2008; Mateo-March et al., 2012), highlighting cycling's ever growing popularity.

1.2 Cycling Components

Cycling performance can vary depending on the optimisation of physiological, psychological, biomechanical parameters, and wider environmental conditions (Turpin & Watier, 2020). Some important components to a cyclist's performance include strength, power, speed, balance and coordination, and endurance, respectively.

Increasing a cyclist's strength is an important component to their performance (Vikmoen & Rønnestad, 2021). For example, having strong calves (soleus) allows for a greater force to be applied to the pedals (Sanderson et al., 2006). Furthermore, strong legs (including the gastrocnemius and quadriceps) contribute to a greater cycling economy, meaning the load required to pedal is reduced and muscle fibres are more efficiently activated (Arkesteijn et al., 2016; Loveless et al., 2005). Additionally, strong gluteus muscles allow cyclists to generate greater forces and maintain a greater pelvic stability (Parsons, 2010). Importantly, strength training develops a cyclists slow-twitch muscle fibres, which enhances their resilience to fatigue and enabling them to sustain higher power outputs for longer periods (Chromiak & Mulvaney, 1990; Plotkin et al., 2021).

Power or power output, measured in watts (W), can be used as a direct measure of exercise intensity during cycling (Vogt et al., 2006). It represents the force generated by the working legs when pushing the pedals (Turpin & Watier, 2020). Cyclists can monitor their power output using a power meter, which measures their torque and cadence (Lucia et al., 2001; Passfield et al., 2017; Spagnol et al., 2013). An increased amount of muscle fibres enables cyclists to generate more power when pedalling, enhancing their ability to accelerate to higher speeds, which could be critical in competitive events (Parkin & Rotheram, 2010). Importantly, optimal performances occur when speed increases to a point where the power demand can match the power supply from energy stores (Olds et al., 1993). However, in events such as off-road cycling, a lower speed can be beneficial as it allows cyclists to coordinate through rough terrains more efficiently.

Balance is the ability to stay upright and in control of body movement (Ragnarsdottir, 1996), whilst coordination is the ability to control more than one muscle simultaneously

(Poprzecki et al., 2009). Since cycling is a repetitive exercise, both balance and coordination are essential for maintaining an effective force applied to the pedals (Blake et al., 2012). However, balance and coordination can be affected by a cyclist's pedals, shoes, cadence, and fatigue (Hug & Dorel, 2009). These factors could influence duration, direction, and magnitude of force being applied to the pedals, which affects a cyclist's power output (Blake et al., 2012). If a cyclist has sufficient balance and coordination, they could better maintain their strength and optimise their performance across other components to their cycling.

Endurance is important as it enables cyclists to exercise at higher capacities for longer durations (Bosquet et al., 2002). It is also one of the most reported indicators of a cyclist's performance, such as maximum oxygen uptake (VO_2max), lactate threshold, ventilatory threshold, and exercise economy (Bentley et al., 2001; Hoogeveen et al., 1999; Lucia et al., 2002; Rønnestad & Mujika, 2014). The maximum rate at which oxygen can be taken up and utilised during exercise is known as VO_2max, serving as an indicator of an individual's aerobic capacity (Bassett & Howley, 2000; Ranković et al., 2010). Lactate threshold is one of the most important predictors of endurance performance as it indicates any changes in endurance capacity by marking the intensity at which lactate begins to accumulate (Faude et al., 2009). Additionally, the lactate threshold can be used to indicate an athlete's aerobic capacity or VO2max, with well-trained endurance athletes being able to produce high VO_2max measures with minimal lactate accumulation (Durocher et al., 2008; Tanaka et al., 1984; Withers et al., 1981). Exercise economy is another powerful predictor of endurance performance as it refers to the volume of oxygen (VO_2) required to perform at a given power output, speed or work rate (Jones & Carter, 2000). Importantly, being adaptable to endurance can reduce the feelings of fatigue and exertion when exercising (Ament & Verkerke, 2009). However, prolonged endurance cycling can lead to the progression of muscle glycogen depletion, which

may alter muscle metabolism (Conlee, 1987). This depletion is likely achieved due to less oxygenated blood being circulated towards the working muscles (Wan et al., 2017), resulting in neuromuscular fatigue ([NMF], Williams et al., 2013).

1.3 Neuromuscular Fatigue (NMF)

Fatigue is a gradual process which includes several complex physiological changes within the muscles (Cairns et al., 2005). It is known as a psychophysical phenomenon which often leaves a sensation of tiredness, leading to a decrease in muscular performance and function (Abbiss & Laursen, 2005; Romani, 2008). It can lead to a failure to maintain a force/power-generating capacity of a muscle or muscle group due to exercise (Grassi et al., 2015; Vøllestad, 1997). This impairment to athletic performance depends on the nature of the exercise causing fatigue, known as task dependency (Enoka & Stuart, 1992). However, this commonly occurs when an athlete has exceeded their maximal efforts, which has progressively occurred during prolonged exercise (Green, 1997). Additionally, fatigue may occur if an athlete is injured, which can lead to a temporary impairment to their exercise capacities however, this can be reversable once recovered (Slobounov, 2008). Despite, the negative connotations associated with fatigue, it can protect muscular fibres from potential damage (Appell et al., 1992).

The two important mechanisms of neuromuscular fatigue (NMF) are peripheral fatigue and central fatigue, respectively (Boyas & Guével, 2011). Peripheral fatigue occurs when the motor units of the peripheral nerves, motor endplates, and muscle fibres change beyond the neuromuscular junction, causing a loss of neuromuscular strength (Gandevia, 2001; Garrandes et al., 2007). This can develop for numerous reasons including a depletion

of glycogen available in the muscle, changes in electrical properties of the muscle, and the presence of metabolites that interfere with the process of maintaining Adenosine Triphosphate (ATP) levels (Amann, 2011; Bigland-Ritchie et al., 1978; Kirkendall, 1990). Central fatigue affects the proximal motor neurons of the brain and spinal cord (central nervous system), leading to a decreasing neural drive to the neuromuscular junction and in voluntary muscle activation (Gandevia, 2001; Taylor et al., 2016). This can lead to a reduced capability to sustain voluntary muscle contracts and overall cycling performance (Enoka, 1995; Hureau et al., 2016). Furthermore, it has been previously hypothesised that muscle damage can influence central and peripheral fatigue (Endoh et al., 2005). Muscle damage can result in swelling, a reduction in range of joint motion, and delayed onset muscle soreness that can peak between 24 to 48 h post exercise (Clarkson & Hubal, 2002; Newham, 1988).

However, it has been previously reported that regular training at the appropriate intensity and duration can improve an athlete's energy supply (Bytomski, 2018), which can positively influence muscle morphology and contractile function (Theofilidis et al., 2018). This can result in an enhanced resistance to fatigue, therefore improving exercise performance (Egan & Zierath, 2013; Nader, 2006). Additionally, athletes can also enhance their resistance to fatigue through specific dietary strategies, some examples include; (1) timing of carbohydrate (CHO) consumption, (2) staying hydrated, and (3) limiting caffeine intake (Coyle & Coggan, 1984; Hurley et al., 2013; Minshull & James, 2013).

1.4 Nutrition and Cycling Performance

Nutrition is becoming more recognised as a necessary component for optimising a sports performance (Burke et al., 2013), with the use of periodised or planned nutrition

becoming more popular with sports practitioners (Stellingwerff et al., 2019). The aim of these nutritional strategies is to enhance adaptations to exercise and training, which could lead to an enhanced athletic performance (Jeukendrup, 2017). Some specific nutritional methods focus on; (1) intestinal absorption, (2) stomach comfort, and (3), dehydration (Jeukendrup, 2017), with effective dietary strategies not only increasing macronutrients (CHO, fat, and protein), fluids, micronutrients, and/or ergogenic aids, but also timing the consumption and adapting intakes to the different environmental conditions (Lambert & Goedecke, 2003; Lemon et al., 2002; Maughan & Shirreffs, 2008). The importance of individualising dietary advice based on an athlete's training demands, specific sport or personal goals has been recognised (Beck et al., 2015; Jeukendrup, 2014).

CHO's are essential for athletes to fuel their performances and support their immune system (Garthe & Maughan, 2018). This macronutrient is especially crucial for high intensity and/or endurance performances as it becomes the main energy substrate (Noakes, 2000). Importantly, CHO are considered an optimal fuel source for exercise power output, recovery and glycogen resynthesis (Burke et al., 2017). For everyday training, it has been recommended that athletes ingest between 6 to 10 g of CHO per kg of body weight per day with moderate training, with 1 to 4 g per kg of body weight of CHO 1 to 4 h before exercise (Clifford & Maloney, 2015; Mata et al., 2019). During high intensity training, CHO requirements can range between 8 to 10 g per kg of body weight (Pendergast et al., 2011). However, for post-exercise recovery, it has been suggested that consuming between 78 to 90 g per hour ($g \cdot h^{-1}$) of CHO during exercise can delay fatigue, due to sparing hepatic glycogen and enhancing CHO oxidation rate, which aids higher intensity performances and avoiding hypoglycaemia (Cermak & van Loon, 2013; Jeukendrup, 2011; Smith et al., 2013). Most recent literature has suggested that athletes may even be able to consume close to 120 $g \cdot h^{-1}$ (Costa

et al., 2017; Smith et al., 2013; Viribay et al., 2020). Furthermore, during recovery, CHO can aid muscle glycogen resynthesis (Zachwieja et al., 1993). However, the time of ingestion, type of CHO, and quantity of CHO can affect this process (Ivy, 1998; Parkin et al., 1997).

Dietary fats are important as they aid the absorption of some vitamins such as vitamins A, D, E, and K (Booth, 2012; Dawson-Hughes et al., 2015; Jeanes et al., 2004; Papas, 2019; Ribaya-Mercado, 2002). For athletes stored fat (in the form of triglycerides in adipose or fat tissue) can also provide a source of energy, especially in aerobic power outputs (Lowery, 2004; Pendergast et al., 1996). It has theorised that ~1 kg of adipose tissue can be an effective energy supply for ~10 to 20 h (Bjorntorp, 1991). Fat can provide significant energy sources for low aerobic power outputs (> 40%), moderate intensity exercises (~50%), extended periods of exercise, and recovery between exercises (Hawley, 2001; Malatesta et al., 2009; Pendergast et al., 1996). Current guidelines recommend 20 to 35% of your daily total energy intake should come from dietary fats (Jensen et al., 2014), further recommending an avoidance of trans fats and to limit saturated fats to ~10% of total consumption (Bytomski, 2018; DeSalvo et al., 2016).

Athletes need adequate protein intake to provide essential amino acids which their bodies cannot naturally synthesise (Tipton & Wolfe, 2004). The nine essential amino acids include; 1) histidine, 2) isoleucine, 3) leucine, 4) lysine, 5) methionine, 6) phenylalanine, 7) threonine, 8) tryptophan, and 9) valine (Shih, 2003). Protein is vital for the growth and development of new muscle tissues, as well as for repairing muscle fibres damaged through exercise (Huard et al., 2002; Stokes et al., 2018). Furthermore, it aids the production of multiple enzymes and hormones, such as adrenaline, maintaining fluid balance, and regulates blood clotting (Acher, 1960; Kalafatis et al., 1997; Lobo, 2004). The current recommended

protein intake is 0.8 per kg body mass per day for the general population (Trumbo et al., 2002), with endurance athletes aiming for protein intakes of between 1.0 to 1.6 g per kg of body weight per day (Lemon, 2000; Meredith et al., 1989). Furthermore, muscle protein synthesis is upregulated 24 h post endurance exercise due to an increased sensitivity to oral protein intake (Burd et al., 2011; Jäger et al., 2017), making this an ideal time to consume protein to help maintain muscle mass.

Essential dietary components, known as micronutrients, are made of organic substances (fat or water-soluble vitamins), inorganic minerals, and trace elements (Shergill-Bonner, 2013). These micronutrients have numerous functions, including supporting enzyme systems, energy production and cell membranes (Shergill-Bonner, 2013). Some essential minerals include calcium, phosphorus, magnesium, potassium, chloride, and sodium (Fairweather-Tait & Cashman, 2015). However, micronutrients cannot be synthesised by the human body and therefore must be obtained from ether dietary sources, gut microbiota, or oral supplements (Godswill et al., 2020; Silverman et al., 2009; Wan et al., 2017).

Inadequate rebalances of CHO, protein, fluids, and electrolytes can hinder an endurance athlete's recovery and future performance (Beck et al., 2015). For example, consuming CHO within two-hours post exercise has been considered as beneficial towards maximising muscle glycogen synthesis rates (Ivy et al., 1988), with further benefits seen when consuming four large CHO meals within 24 h post exercise (Burke et al., 1996). Furthermore, consuming around 20 g of protein post-endurance cycling is considered sufficient to maximise muscle protein synthesis, with protein consumption increasing synthesis rates threefold 45 to 90-min afterwards (Atherton et al., 2010; Rowlands et al., 2015).

Rebalancing fluid and electrolytes should include both water and sodium (Shirreffs et al., 2004), with previous literature recommending a total of 150% of the volume of fluids lost through sweat should be consumed to support rehydration (Maughan et al., 1996; Shirreffs & Maughan, 1998, 2000). Alternatively, the consumption of a sodium-based meal and/or snack alongside water is a sufficient hydration strategy (Park et al., 2012). However, the use of an electrolyte solution, instead of plain water, has been theorised as a more rapid hydration rebalance strategy (Maughan & Shirreffs, 1997).

When cyclists are injured, their dietary choices need to be modified to facilitate the role of recovery and rehabilitation (Smith-Ryan et al., 2020). For example, 3 to 5 g per kg of body weight, or approximately 55% of total calorie intake, should be from complex CHO (Thomas et al., 2016). Furthermore, to preserve muscle mass, cyclists should aim for approximately 2 to 2.5 g per kg of body weight of protein per day (Mettler et al., 2010; Tipton, 2015). There is theoretical evidence that some nutrients can aid an injured athlete however, the evidence has varying results (Tipton, 2015). These include creatine at 0.3 g per kg of body weight per day for 3 to 5 days or 20g per day for 5 to 7 days (Wax et al., 2021), vitamin C at 500 mg per day (Braakhuis, 2012), and vitamin D at 2000 to 5000 IU per day (Miraj et al., 2019).

Ergogenic aids are substances or techniques that can be used with the aim of enhancing sport performance (Burke et al., 2000; Thein et al., 1995). They commonly contain nutrients which are greater than what is typically provided in foods (Burke & Read, 1993). Due to challenges in maintain optimal dietary habits or sourcing specific nutrients, the use of supplements is becoming increasingly popular amongst athletes (Matusiak-Wieczorek et al., 2023; Sparks et al., 2018).

1.5 Supplements and Cycling Performance

Dietary supplements are intended to complement one's regular diet and have been indicated in having performance-enhancing effects (Bishop, 2010). They can be in a variety of forms such as capsules, tablets, liquids, powders, soft gels, and drinks (Froiland et al., 2004). Some popular supplements that have indirect benefits to a cycling performance include creatine (Van Schuylenbergh et al., 2003), sodium bicarbonate (Higgins et al., 2013), caffeine (McNaughton et al., 2008), CHO (Rauch et al., 1995), and omega-3 fatty acids (omega- 3; James et al., 2020).

Creatine supplementation has been indicated to improve maximal repetitive power and lower-body strength (Izquierdo et al., 2002). Previous research has found that creatine can provide a rapid energy turnover which delays the onset of fatigue (Bemben & Lamont, 2005; Kreider et al., 2017). It is beneficial for sports that require repeated short bouts of high-intensity exercise, such as squash, football, and sprint cycling (e.g. short-track, Crisafulli et al., 2018; Bemben & Lamont, 2005; Mujika et al., 2000; Romer et al., 2001). However, creatine can commonly result in gastrointestinal upsets, including cramping (Mesa et al., 2002; Poortmans & Francaux, 2000; Vandebuerie et al., 1998).

Sodium bicarbonate is effective at counteracting muscle acidity levels, which helps reduce fatigue during intense exercise (Burke et al., 2000; Shelton & Kumar, 2010). This supplement is beneficial for events lasting 1 - 7 min such as 400 m -1500 m running, 100 m – 400 m swimming, and sprint cycling (Shelton & Kumar, 2010). However, sodium bicarbonate can cause gastrointestinal discomfort, which can vary from mild to extreme discomfort, consequently negatively impacting performances (McNaughton, 1992; Price & Singh, 2008; Siegler et al., 2012). The degree of discomfort depends on the dose, timing of ingestion and

the individual response (Carr et al., 2011; Siegler et al., 2012). Therefore, it is recommended that sports practitioners approach this supplement with caution due to the large degree of subject-variability (Siegler et al., 2012).

Caffeine has demonstrated its ability to increase energy substrates during exercise by acting as a glycogen saver (Laurent et al., 2000). It benefits high-intensity endurance sports, such as running or cycling (Southward et al., 2018; Wang et al., 2022). However, if caffeine is not closely monitored, it can cause poor sleeping patterns (Young et al., 2020), nausea (Wilson, 2019), and headaches (Shapiro, 2008) which can hinder a cycling performance (Amin et al., 2018; Marshall & Turner, 2016). Additionally, caffeine dependence and withdrawal (occurs within 12- 48 h after last ingested) can occur, with symptoms including depression, irritability, and a decrease in alertness and productivity (Griffiths & Woodson, 1988; Jenkinson & Harbert, 2008; Juhn, 2003; Juliano et al., 2012; Juliano & Griffiths, 2004)

As previously mentioned in Chapter 1.4, CHO are an essential energy source (Burke et al., 2017; Garthe & Maughan, 2018)**.** However, CHO supplements are known to have a more rapid absorption rate in comparison to food sources (Mata et al., 2019). The different sources of CHO supplements include; (1) drinks, (2) bars, (3) gels, and (4) jellybeans (Campbell et al., 2008). An advantage of these supplements is that it can benefit a variety of sports, such as endurance, intermittent, and resistance disciplines (Baker et al., 2015; Haff et al., 2003; Vandenbogaerde & Hopkins, 2011). The CHO supplement intake guidelines should correspond to what was previously stated in Chapter 1.4. However, CHO supplementation can lead to digestive discomfort if highly concentrated (Mata et al., 2019; Pfeiffer et al., 2009). Therefore, to reduce these symptoms, it is recommended to avoid dehydration (de Oliveira et al., 2014).

Omega-3 is a polyunsaturated fatty acid, which is characterised by their hydrocarbon chains with carboxyl group attached, commonly found in fat and oil molecules (De Carvalho & Caramujo, 2018; DeFilippis & Sperling, 2006; Zambiazi et al., 2007). They have been theorised to improve training adaptations and exercise recovery by potentially enhancing the recovery from exercise induced muscle damage by increasing the structural integrity of the muscle membrane, which aid the recovery from exercise induced muscle damage (Philpott et al., 2019; Stupin et al., 2019). Furthermore, omega-3 is known for its anti-inflammatory properties, which may reduce muscle damage and perceived muscle soreness, allowing for a faster recovery rate (Armstrong, 1986; Peake et al., 2017; Pyne, 1994). Due to this, omega-3 supplements have increased in popularity among athletes in strength, endurance, and team-based events, respectively (Mickleborough, 2013; Philpott et al., 2019; Thein et al., 1995). Consequently, both amateur and well-trained athletes have sought to utilise omega-3 supplementation with (Thielecke & Blannin, 2020), suggesting that amateur athletes often demonstrate a greater performance benefit. This might be because well-trained athletes may have already reached their optimal physiological limits and therefore do not respond as well (Sandbakk & Holmberg, 2017). However, Thielecke & Blannin (2020) reported that a greater number of previous studies have utilised an amateur population in comparison to well-trained athletes.

1.6 Omega-3 polyunsaturated fatty acids

Omega-3's have at least one carbon-carbon double bond between the third and fourth atom from methyl end of the fatty acid chain (Hulbert, 2021). The most recognisable omega-3s include; Alpha-Linolenic Acid (ALA) with 18 carbon atoms (Stark et al., 2008)

Eicosapentaenoic Acid (EPA) with 20 carbon atoms (Aas et al., 2006), Docosahexaenoic Acid (DHA) with 22 carbon atoms (Narayan et al., 2006), and Docosapentaenoic acid (DPA) with 22 carbon atoms (Miller et al., 2013), respectively (Stark et al., 2016). Collectively, EPA, DHA, and DPA are classified as long chain omega 3's due to the large quantity of molecules (Swanson et al., 2012). The human body can only produce carbon-carbon double bonds after the ninth carbon and therefore must be provided by the ingestion of foods, this is why ALA is considered an essential fatty acid (Insel et al., 2022; Lunn & Theobald, 2006). However, between 8 to 20 % of ALA can be converted into EPA and 0.5 to 9 % of ALA can be converted into DHA via the liver (Stark et al., 2008). As this process is limited, consuming omega-3 rich foods or supplements, which contain EPA and DHA is an efficient method for increasing fatty acid intake (Nguyen et al., 2019).

Omega-3's provide multiple beneficial functions for the human body, impacting multiple physiological process, including aiding the structures of cell membranes (Versari et al., 2008). They can also alter platelet activation by influencing eicosanoids production, creating an antithrombotic effect, producing more efficient wound healing (Gammone et al., 2017; Gammone et al., 2019). They can also regulate blood clotting hormones (Calder et al., 2019), reduce blood pressure (Gebauer et al., 2006), help eicosanoids formation (Simopoulos, 2002), and help with the contraction and relaxation of the artery walls in the heart (Zanetti et al., 2015). If the artery walls are not fully utilised, it may reduce oxygenated blood flow towards any working muscles, resulting in a build-up in lactic acid (Sahlin, 2014; Willis et al., 2018). Furthermore, increase muscle contractions during exercise can elevated blood pressure which may restrict blood flow to the working muscles and contribute to NMF (Degens et al., 1998; Wright et al., 1999). As previously detailed (see Chapter 1.5), the

supplementation/ingestion of omega 3's has been theorised in aiding athletic performance and enhancing recovery, which could therefore improve cycling performance(s).

Chapter 2. Literature Review

Cycling training has been indicated as being one of the most physically demanding sports due to the combination of exercise duration, intensity, and frequency (Jeukendrup et al., 2000), with it now being suggested that the average well-trained cyclist covers between 25,000 to 35,000 km per year (Simonetto et al., 2016). As cycling is continuously growing in popularity, there is a demand for further research into how both athletes and amateur cyclists can optimise the training practices and utilise their nutritional habits to improve performance and recovery.

2.1 Energy Systems

Created in the mitochondria, ATP is generated from the breakdown of food molecules and provides the skeletal muscles with energy to perform a contraction (Alberts et al., 2002; Hargreaves & Spriet, 2020; Martin & Mentel, 2010). The total quantity of ATP stored in a cell is approximately 8 mmol/kg wet weight of muscle; therefore, the cells rely on other sources to supply ATP for their function (Baker et al., 2010). The ATP process has three major roles in muscle contractions: generating force against adjoining actin filaments through the cycling of myosin cross-bridges, pumping calcium ions (Ca^{2}+) from the myoplasm toward the sarcoplasmic reticulum and transporting sodium (Na+) and potassium (K^{+}) ions across the sarcolemma (Barclay, 2011).

Theoretically, the greater amount of ATP production, the greater the performance (Clark et al., 2010). In well-trained cyclists, the rate at which ATP production increases is

significant at 1,000-fold compared to rest due to their enhanced metabolism (King et al., 1983; Zhang et al., 2019). This demonstrates that the energy supply needs to be correctly utilised and is a critical factor in an endurance athletes' performance (Sahlin, 2014). For a well-trained cyclist, the most common energy systems supporting these energy demands are: (1) the phosphocreatine (PCr) energy system, 2) anaerobic glycolysis system and 3) the aerobic energy system (Boulay, 1995; Gastin, 2001 [see Figure 1]).

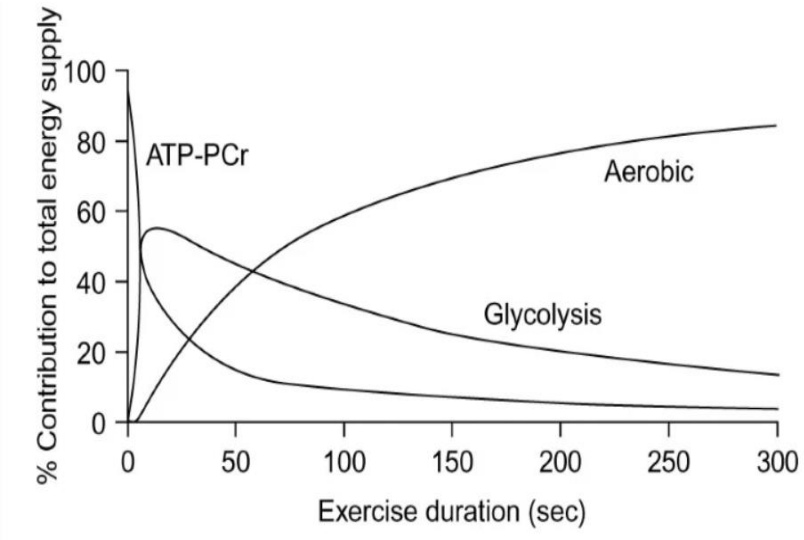

Figure 1. Energy systems used during exercise duration (adapted from Gastin, 2001).

The ATP-PCr pathway: The PCr energy system uses creatine phosphate to produce ATP anaerobically and is the most powerful source of ATP (Gastin, 2001). However, it lasts for less than ~10 s of intense cycling (Sumner, 2016). Research from Trump and colleagues (1996), investigated the importance of muscle PCr in maintaining power output and velocity during an intermittent maximal cycling test (30 s; 100 revolutions per minute, with a four-minute

rest between bouts). Muscle biopsies from the vastus lateralis were analysed before and after bout three. This study identified that PCr contributes to ~15 % of the total ATP production during a third bout of 30 s maximal isokinetic cycling, with most of the contribution occurring within the first 15 s. Therefore, this energy system is most beneficial during maximal efforts due to its rapid ATP production, for example fuelling a maximal effort sprint for a well-trained cyclist (Cheung & Zabala, 2017).

The glycolysis pathway: Intense cycling efforts that last approximately 30 s to 3-min primarily utilise the anaerobic energy system (Gastin, 2001). This system does not require oxygen and converts glucose into ATP (Medbø & Tabata, 1993). The rate at which the anaerobic system produces ATP is critical in responding to the high energy demands required to produce high power outputs (Gastin, 2001). Medbo and colleagues (1989), aimed to investigate the amount of anaerobic energy released when cycling to exhaustion. A total of 17 male cyclists were recruited (VO_2max 52 ± 1 ml·kg·min^{-1}) and separated into three groups, cycling for either (1) 30 s, (2) 1 minute, or (3) 2 to 3 min at a consistent power on a cycle ergometer. Muscle biopsies were performed before and ~10 s after exercise to allow for analysis of lactate. This study found that ATP was produced at a rate of 58 ± 2 mmol/kg wet muscle mass. Suggesting that this might be the maximum anaerobic energy released during cycling. A well-trained cyclist is likely to use the glycolysis system when they are close to their threshold during a steady state cycle (SSC) or during sprinting efforts (Douglas et al., 2021; Ghosh, 2004; Impellizzeri & Marcora, 2007).

The aerobic pathway: The primary source of energy for a cyclist is the aerobic energy system (Carmichael & Rutberg, 2012), which uses oxygen to produce energy for extended periods (Gastin, 2001). This system re-synthesises ATP through the metabolism of glucose,

stored fats and stored CHO (Clark et al., 2010). This system is most beneficial for the demands of intense exercise; however, it does not produce ATP as rapidly as the other anaerobic systems. Instead, it relies on the capacity to continually produce ATP through three stages (Baker et al., 2010). The first stage is aerobic glycolysis, which produces two ATP molecules per molecule of glucose (Chandel, 2021). Next the Krebs cycle synthesises ATP and produces two molecules of ATP (Draper, 2014). This is also an important component in the synthesis of substances such as amino acid and fatty acids (Weinman et al., 1957). Finally, the electron transport chain produces 34 ATP molecules and overall, the aerobic energy system produces a total of 38 ATP molecules (Loehr et al., 1985). Due to its ability to sustain energy production for longer durations, it is considered the most efficient method for generating energy (Cahill et al., 1997; Soldavini, 2019). Therefore, cyclists could theoretically delay the onset of fatigue due to the presence of oxygen (Bogdanis, 2012). However, if a cyclist cannot meet the demand for more ATP through an increase of oxygen delivery, it can result in an imbalance of metabolic homeostasis, leading to NMF and a subsequent decline in performance (Kent et al., 2016).

In summary, it has been well documented that well-trained cyclists primarily rely on ATP during exercise. However, to enhance performance, they must efficiently utilise their energy systems (ATP-PCr, glycolysis, and aerobic metabolism). This understanding informed the development of the experimental protocol, which aimed to assess whether well-trained cyclists can efficiently utilise their energy systems to sustain an endurance-focused protocol and mitigate NMF.

2.2 Time Trial (TT) Distances

Time trials consist of participants completing a quantity of work over a distance as quickly as possible (Holm et al., 2004). They are commonly utilised in endurance sports and/or in sports to measure an individual's aerobic capacity, muscular endurance, and speed (Coakley & Passfield, 2018; Jacobs et al., 2011; Lundquist et al., 2021). In the context of cycling, riders may race across a range of common distances, which can be categorised as; short/prologue (~5 km), medium (~20 km), or long (~60 km) in duration, with short and medium distances typically raced in Grand Tour stage-races or even in the Olympic games (Atkinson et al., 2007). However, completing a TT in a laboratory-based setting creates a controlled environment to perform a more comprehensive and standardised evaluation of power output, performance time (s), and other physiological and psychological variables (Smith et al., 2001). These variables are important indicators of cycling TT performance (Faria et al., 2005). However, during a cycling TT performance, participants are likely to become fatigued due to factors such as energy depletion (Coyle et al., 1986), reduced muscular activation (Gandevia, 2001), cardiorespiratory stress (Amann et al., 2007), and muscular tissue damage (Appell et al., 1992), which can result in a reduction in power output in the working muscles. When determining TT protocols, practitioners should be aware that results can differ between speed, time, and power data (Sparks et al., 2016).

Research by Dantas and colleagues (2015), investigated the aerobic endurance fitness changes in 20 well-trained cyclists (VO_2max 360.5 ± 49.5 W), during a 5 km TT. The results of this study suggest that a 5 km TT is a valid test to determine the aerobic endurance fitness of well-trained cyclists, as changes in performance could be found through absolute (± 17.7 $W \cdot kg^{-1}$) or relative power output (± 0.3 $W \cdot kg^{-1}$), time to complete (± 13.4 s), and the average

speed (± 1.0 $km \cdot h^{-1}$), respectively. Although, they determined this was a valid test for measuring aerobic endurance fitness in this population, it did not demonstrate reliable physiological and neuromuscular changes, leading the researchers to see no reason to investigate these variables. However, this has been the only study to date to determine the validation of a 5 km TT, suggesting that further research should focus on longer TT distances. Thomas and colleagues (2012), investigated the reproducibility of performance, perception of exertion and physiological responses during three sets of 20 km TTs in well-trained cyclists (n = 17; VO_2max = 4.78 ± 0.36 $L.min^{-1}$ kg^{-1}). These findings highlight the reproducibility of 20 km TTs in this population, with the typical error of mean power between TT1 and TT2 to be 1.6 % (1.2 to 2.3 %) and between TT 2 and TT 3 to be 2.2 % (1.6 to 2.6 %), respectively. Furthermore, the mean time to complete each of the TTs (p = 0.59; TT 1 31. 99 ± 0.99; TT 2 31.98 ± 1.05; TT 3 31.90 ± 1.02 min), failed to show any significant differences between each TT, finding that the high reproducibility of the TT performance (typical error range 1 to 4 %). However, Hopkins and colleagues (2001) suggested that repeated assessments usually enhance in performance due to participants becoming well-practiced, with Micklewright and colleagues (2010), further suggesting that highly experienced competitive cyclists are good at pacing this type of effort.

Balmer and colleagues (2000), investigated whether peak power could be used to predict performance during a 16.1 km TT. In the first part of the study, nine participants (age 31.3 ± 7.4 years; peak power output of 434.3 ± 31.8 W) performed three VO_2max tests, where work rate was increased every minute by 5.0 ± 0.2 % of peak power output until volitational exhaustion. In the second part, 16 cyclists (age 43 ± 15 years; peak power output of 311 ± 61 W) completed one maximal aerobic power test, using the same protocol, followed by the 16.1

km TT. The results from this study identified a significant relationship between peak power output and 16.1 km TT power output (r = 0.99 [*very strong*]; p < 0.001). However, the relationship between peak power output and 16.1 km time was not significant, yet there was a *moderate* correlation (r = 0.46; p > 0.05). Collectively, these findings suggest that peak power output is a stronger measure of endurance performance during a 16.1 km TT than time to complete the TT. Additionally, Sparks and colleagues (2016), found that the trained cyclists who performed the 16.1 km TT slower, had a lower reliability rate (coefficient of variation [CV] range 1.3 - 3.2 %) in comparison to the cyclists who performed it faster (CV range 0.7 - 2.0 %).

Time trials are widely used by endurance athletes to assess performance variables. These findings support the validity of TTs in evaluating a well-trained cyclist's aerobic capacity, muscular endurance, and speed. However, there is limited research which has focused specifically on endurance cyclists. This gap in the literature contributed to the development of the experimental protocol, supporting the reliability of TTs as a performance assessment tool.

2.3 Neuromuscular Fatigue (NMF) in Well-trained Cyclists

It has been suggested that high-intensity, prolonged cycling can cause progressive muscle glycogen depletion (Coyle et al., 1986; St Clair Gibson et al., 2001). Due to this depletion, it is expected that cyclists will experience a form of NMF during an endurance performance, with Lepers and colleagues (2002), finding that the first signs of NMF can be present after the 1st h of endurance exercise. Furthermore, signs of exercise-induced muscle

damage can typically occur within the first 24 h and can last between five to seven days (Cleak & Eston, 1992).

Knaflitz and Molinari (2003) have suggested that cyclists prominently feel symptoms of fatigue in the rectus femoris, gastrocnemius and bicep femoris muscles, with prolonged cycling effecting muscle strength capacities associated with changes in properties of leg extensors (Lepers et al., 2001). Research from Lepers and colleagues (2000), investigated the presence of NMF in eight well-trained cyclists or triathletes (age 26 ± 4 years; VO_2max 338 ± 56 ml·kg·min^{-1}; weekly training distance 180 ± 80 km) after a 2 h prolonged cycling at a power output of ~ 60% maximal aerobic power (VO_2 338 ± 56 ml.kg.min^{-1}). Muscular strength in the quadriceps was determined using isokinetic dynamometry following a 2 h prolonged cycling protocol (~ 60% maximal aerobic power). The results from this study identified a significant reduction ($p < 0.05$) in leg muscular capacity and peripheral contractile mechanisms, as the root mean square activity was lower at each angular velocity from pre- to post-prolonged cycling protocol in the vastus medialis (-60° s^{-1} to 120° s^{-1}) and vastus medialis (120° s^{-1} to 240° s^{-1}) muscles. These findings are supported by Sahlin and Seger (1995), who investigated the effects of prolonged exercise on the contractile properties of human quadriceps in seven healthy male subjects (aged 27 years, VO_2max 59.7 ml·kg·min^{-1}). Participants were required to cycle at ~75% maximal aerobic power until volitional exhaustion, with data showing a significant reduction ($p < 0.05$) in maximal voluntary concentric forces from pre- to post-exercise (559 to 145 N; 74% [SEM 4] decrease), with eccentric forces also decreasing significantly (733 to 146 N; 80% [SEM 4] decrease). Collectively, these studies suggest that high-intensity, prolonged cycling (> 60% maximal aerobic power) leads to significant

reductions in muscular strength and neural input to the quadriceps, contributing to NMF during endurance cycling performance.

During high-intensity cycling, muscle glycogen is depleted which alters skeletal muscle metabolism, causing peripheral fatigue (Coyle et al., 1986; St Clair Gibson et al., 2001). Even though peripheral fatigue is dominant in the early stages of exercise (~ 6 min), it is not the most significant factor of fatigue during high-intensity cycling (Bigland‐Ritchie et al., 1982). The primary cause of fatigue is the build-up of metabolites such as lactate in the working skeletal muscles (Abbiss & Laursen, 2005). Lactate, or lactic acid is the by-product of anaerobic energy production (De Backer, 2003). When muscles are pushed beyond its aerobic threshold, lactate accumulates and inhibits the metabolic mechanisms within the muscular cells, resulting in a burning sensation (Rabinowitz & Enerbäck, 2020; Sembulingam & Sembulingam, 2012). Some symptoms of lactic acid include tiredness and increased fatigue (Bettelheim et al., 2012). Research by Stepto and colleagues (2001), investigated the metabolic demands of intense aerobic interval training (8 x 5-min work bouts at 86 ± 2 % of VO_2peak with 60 s recovery). Seven competitive cyclists (age 26.9 ± 5.4 years; VO_2peak 5.14 ± 0.21 $L \cdot min^{-1}$), were required to complete the exercise protocol with muscle biopsies taken from the vastus lateralis, pre- and post- to quantify the metabolic demands of the whole training session rather than a single bout of work. The results from this study found a significant increase in muscle lactate (6.2 to 32.7 mmol/kg dry mass; $p < 0.01$), which suggests the symptoms of lactate acid accumulation begin once cycling durations exceed 30 min (Schillings et al., 2003; Woodward & Debold, 2018).

Prolonged endurance cycling has been shown to induce NMF, significantly reducing quadriceps function and leading to lactate accumulation, which may impair performance in

well-trained cyclists. However, the potential roles of omega-3 supplementation in mitigating these effects remain unclear. This study aimed to determine whether a fixed dosage of 1600 $mg \cdot day^{-1}$ of omega-3 for 56-days (8-weeks) could reduce the presence of NMF following a high-intensity endurance exercise protocol in well-trained cyclists.

2.4 Measurements of Fatigue

To measure fatigue in athletes, numerous tests can be performed. A widely used example of this is through the use of self-reporting measures (e.g. questionnaires) that quantify subjective fatigue levels, focusing on how athletes perceive their fatigue levels (Marcora et al., 2009). One of the most widely used methods for this is via the Borg scale (1967 & 1982), which uses the rating of perceived exertion (RPE). The original method (1967), asks the user to rate their perceived effort on a scale of 6 (no exertion) to 20 (maximal exertion) or the modified Borg which uses a 0 (nothing) to 10 (maximum) scale (1982). This scale is derived from the psychophysical process of combining sensation of physical stress, discomfort, and fatigue during intense exercise (Haddad et al., 2014). Research by Garcin and colleagues (1998) investigated the RPE response, using the original Borg scale, during cycling exercises at a constant power output in male cyclists (n = 10). This study found that the relationship between RPE and % of exhaustion time were similar (not significantly different, $p > 0.05$) for exercises at 60 and 73% maximal aerobic power, suggesting that RPE is a subjective estimation of the hardness of exercise rather than intensity. Furthermore, finding that RPE should not be used as the predictor of point of self-imposed exhaustion. Indicating that while the original RPE can provide valuable insights into an athlete's perceived exertion, it may not always accurately predict exhaustion levels.

Another method in analysing fatigue is through blood or saliva to monitor changes in biochemical, hormonal, and immunological markers (Haff & Triplett, 2015; Twist & Highton, 2013). The most recognised biochemical markers for fatigue from ATP production include blood lactate and Interlukin-6 (IL-6; Finsterer, 2012). Some accurate hormonal markers of fatigue include thyroid hormones and the cortisol to testosterone ratio (Gleeson, 2002; Nicoll et al., 2018). Furthermore, creatine kinase (CK) and Insulin-like Growth Factor 1 (IGF-1) are also well-regarded indicators of fatigue in athletes (Alba-Jiménez et al., 2022; González-Badillo et al., 2015). Hecksteden and colleagues (2016), investigated blood-borne fatigue markers during and after discipline specific training camps in 73 competitive athletes (cycling n = 28; team sports n = 22, strength n = 23). Blood markers were collected after an initial resting phase, a six-day induction to fatigue protocol, and following a two-day recovery period. The results from this study identified that IGF–1 level significantly decreased ($p < 0.001$) from the resting phase to post-fatigue protocol (–56 ± 28 ng/ml) and post-a 2-day recovery period (53 ± 29 ng/ml). These findings therefore reflect the metabolic aspect of fatigue associated with endurance training.

Although useful, blood-markers can display a level of inter-individual variability in measures of fatigue, which suggests that an individualised interpretation approach is recommended (Hecksteden et al., 2016; Thorpe et al., 2017). In light of this suggestion, Hough and colleagues (2021) investigated the reliability of salivary cortisol and testosterone to indicate overtraining status. A total of 23 active males (VO_{2peak} 50.9 ± 7.6 $ml \cdot kg \cdot min^{-1}$) were recruited and required to complete a 30-minute repeated high-intensity cycling protocol (consisting of altering blocks of 1-min at 55% into 4-min at 80% power at VO_2max). The results from this study identified a significant time effect ($p < 0.001$) in salivary cortisol produced as

a result from the cycling protocol. However, the authors expressed some concerns regarding the hormonal variability seen during the exercise protocol. The variations of salivary concentrations were ~27% cortisol and ~14% testosterone, which were lower than previously reported research by Hough and colleagues (2015). This suggests that while salivary cortisol is a reliable marker for detecting changes in response to high-intensity exercise, individual variability needs to be carefully considered when interpreting the results. These results suggest that while blood and saliva markers can be useful for measuring fatigue, their high level of inter-individual variability increases the risk of practitioners drawing incorrect conclusions (Li et al., 2019).

The most valid and reliable tool to measure NMF in athletes is through the use of a countermovement jump (CMJ), which assesses lower body power (Garrett et al., 2019). Research by Gathercole and colleagues (2015), compared the capacity of different jump (6 x 6 CMJ, squat jump, drop jump) and sprint field tests (20 m sprint test) to detect NMF in collegiate level team-sport athletes (n = 11). The data from this study identified that the CMJ tests had the lowest degree of variability (CV 3.0 ± 1.1%), compared to the next best test (squat jump 3.5 ± 1.6%), which demonstrates the high-level of reproducibility. Even though cycling involves minimal stretch-shortening cycles and no variation of jumping, CMJ's are still valuable as they indirectly measure lower-body force, making them an ideal performance indicator for a cyclist (Laffaye et al., 2014; Sánchez-Jiménez et al., 2023). Furthermore, Lewis and colleagues (2022), investigated the reliability of a CMJ for measuring muscle contractile properties in elite sprint (n = 8) and endurance cyclists (n = 8). This study identified that jump heights were significantly different (p = 0.01) between the groups, with the sprint cyclists achieving 42.85 ± 7.54 cm and the endurance cyclists achieving 33.00 ± 6.21 cm. Even though

this is the first study to determine CMJ reliability with an endurance cyclist population, its results suggest that CMJ can effectively measure muscle functions within various cycling disciplines.

In summary, sport and exercise scientists have several tests available to measure fatigue in athletes, including RPE, blood and saliva markers, and CMJ protocols. However, the current findings suggest that CMJ protocols have the most scientific support for assessing muscular function in cyclists. Based on this evidence, the experimental protocol was developed to further investigate whether a CMJ protocol can be used and serve as a reliable indicator of NMF in well-trained, endurance cyclists. Additionally, some athletes have started to implement dietary changes to reduce the onset of fatigue (Jurasz et al., 2022). For example, it has been previously hypothesised that the use omega-3 supplementation may improve muscular function and thereby reduce NMF (Gammone et al., 2019).

2.5 Omega-3 supplementation and Well-trained Cyclists

As previously mentioned (see Chapter 1.5), omega-3 supplementation has demonstrated various benefits relating to exercise performance and recovery, which may be of interest for well-trained endurance athletes. Of particular interest may be omega-3's due to their anti-inflammatory properties (Li et al., 2005), which reduce the production of prostaglandins via the cyclooxygenase-2 pathway (Lim et al., 2009). Furthermore, omega-3 can influence reactive oxygen species (ROS) by increasing levels to counteract inflammation and later delaying ROS production, allowing for oxidative stress and muscle recovery to be manageable (Gammone et al., 2019; Heshmati et al., 2019). In addition to this, omega-3's can

also alter the fatty acid composition of cell membranes, increasing the membrane fluidity, facilitating a more efficient oxygen delivery and cellular function, which is beneficial for enduring the physical stresses of endurance exercise (Andersson et al., 2002; Kamada et al., 1993). Moreover, omega-3 is linked with energy availability as increases in fatty-acid transport proteins, can create greater ATP production, which in turn could enhance aerobic respiration and reduce lactate accumulation during prolonged exercise (Alghannam et al., 2021; Clavel et al., 2002). Omega-3's can also reduce inflammation (Mickleborough, 2013; Shei et al., 2014), which can facilitate a faster recovery from muscle damage caused by prolonged training (Armstrong, 1986; Peake et al., 2017). Collectively, this reenforces the potential benefits athletes may experience when consuming adequate omega-3 in their diet or via additional supplementation (Pyne, 1994). Even though, omega-3 supplementation has been associated with improved endurance performances, the current evidence lacks consensus (Da Boit et al., 2017; Philpott et al., 2019). Due to these aforementioned potential benefits, this study aims to contribute to the understanding of how omega-3 supplementation affects endurance performance and the manifestation of NMF in endurance cyclists.

The best dietary sources of EPA and DHA is fatty fish, known for their high marine omega-3 content (Innes & Calder, 2020). Some examples include mackerel (Lee et al., 2009), salmon (Jensen et al., 2012), and herring (Moss, 2016). According to UK guidance, adults should consume two portions of oily fish (~ 70 g per portion) per week (Sacn, 2004). This is just above guidelines set by the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO), who suggest a daily consumption of ~200 to 250 mg per day (FAO, 2010). However, the modern Western diet often does not meet these omega-3 guidelines (Innis, 2014). As data from the National Diet and Nutrition Survey Rolling

Programme (UK) have shown, indicate that from 2016/17 to 2018/2019, only 25% of adults aged 19 to 64 years and 17% of adults aged 65 years and over, meet the government guideline for fatty acid intake (Public Health England, 2019). Due to this, omega-3 supplements are often recommended, with some examples including krill oil and cod liver oil, respectively (Da Boit et al., 2015; Hansen et al., 2021; Lewis et al., 2020). Dietary supplements can contain omega-3 in different forms, such as triglycerides, fatty free acids, ethyl esters, re-esterified triglycerides, and phospholipids (Burri et al., 2012; Sharma & Kundu, 2006). However, a vegetarian alternative includes products only containing algal oil (Lane, Derbyshire, et al., 2014).

The prevalence of dietary supplement consumption among athletes is estimated to range between 40 to 100%, depending on the type of sport, level of competition, and definition of supplement use (Daher et al., 2022; Garthe & Maughan, 2018). It is widely assumed that both amateur and professional athletes fail to meet their dietary macronutrient and micronutrient goals, particularly in omega-3 (Baranauskas et al., 2015; Thielecke & Blannin, 2020). Despite current estimates suggesting that ~85% of elite athletes use at least one sports supplement (Maughan et al., 2007; Shaw et al., 2016), there is currently no data pertaining to the consumption of omega-3 supplements among well-trained athletes. Additionally, the optimal dose of omega-3 for athletes remains unclear (Philpott et al., 2019). This gap in knowledge informed the development of the experimental protocol, which aimed to determine the optimal dosage of omega-3 (by using a fixed dose of 1600 $mg \cdot day^{-1}$ for 56-days/8-weeks) for well-trained endurance athletes.

2.6 Omega-3 Supplementation and its Effects on Exercise Performance

The side effects of omega-3 supplementation are commonly regarded as mild (Ramprasath et al., 2013), with some examples including bad breath (Freeman & Sinha, 2007) and gastrointestinal symptoms such as heartburn (Bays, 2006). However, these side effects can be reduced if consumption follows the recommendations set by the European Food Safety Authority (EFSA). They recommend that omega-3 is safe to consume if the total daily intake (including both supplements and food sources) does not exceed 5,000 mg per day EFSA (2012). Furthermore, omega-3 supplements are also safe to be consumed in combination with other supplements, such as whey protein and vitamin E (Atashak et al., 2013; Philpott et al., 2018).

Research by Black and colleagues (2018), investigated whether or not adding omega-3 to a protein-based supplement would reduce muscle soreness and the maintenance of explosive power in professional rugby players (age 22 ± 2 years, *n* = 20). Participants consumed either a 200 ml beverage containing protein (15 g) and 1546 mg of omega-3 or the same 200 ml beverage without the omega-3, twice daily for 35-day/5-weeks. Furthermore, participants were asked to perform three CMJs (best score being recorded) at seven different timepoints (baseline, day-5, day-12, day-16, day-19, day-22, and day-35) throughout the supplementation period. Additionally, participants completed a subjective muscle soreness and wellness questionnaire, and a Likert scale questionnaire regarding their sleep, fatigue, stress, and mood (scores ranged between 1 to 5, with 5 indicating “no soreness”), with blood samples taken at baseline, day 19 and day 35, respectively. Blood samples were analysed for plasma, erythrocyte, and buccal cell lipids. The results from this study identified a beneficial effect from baseline to day-35 in the omega-3 (−3.8 ± 21.7 %) group, compared to the placebo

(−19.4 ± 11.2 %) group. Furthermore, results from that muscle soreness and wellness questionnaire identified a significant reduction ($p < 0.05$) in perceived muscle soreness from baseline to day-35 in the omega-3 group, compared to the placebo group ($p > 0.05$). These findings suggest that the inclusion of omega-3 was an effective strategy in reducing lower body muscle soreness.

Currently, there is a lack of consensus to the optimal dosage and duration of omega-3 supplementation (Anthony et al., 2023; Hilleman et al., 2020). For example, James and colleagues (2020), aimed to investigate if omega-3 supplementation could improve HR, RPE, and VO_2 during a 15 min TT following a cycling protocol (45-min of preload at 70% Wmax) with 10 cyclists ($n = 10$; VO_2max 54 ± 5 $ml \cdot kg \cdot min^{-1}$). Participants were instructed to consume ether an omega-3 (5.7 $g.day^{-1}$ of EPA and DHA) or an olive oil placebo (6 $g.day^{-1}$) supplement, with a 4-week wash-out period, after which participants received the opposing supplement. The results from this study identified that there were no significant ($p = 0.7$) changes in VO_2max between the pre- (2.91 ± 0.44 $L \cdot min^{-1}$) or post- supplementation period (2.82 ± 0.42 $L \cdot min^{-1}$). Furthermore, there were no significant ($p = 0.07$) changes in TT performance from the pre- to post- supplementation period (239 ± 34 to 243 ± 33 W). The authors concluded that future research should implement a higher and/or harder intensity cycling protocol due to the population being well-trained. A literature review by Thielecke and Blannin (2020), investigating the effect of omega-3 supplementation to a sports performance, identifying 30 articles using a sporting population. This study found that the more consistantly favourable outcomes of omega-3 supplementation are likely to occur after a six-to-eight-week period at dosages ranging between 1.5 to 2.0 $g \cdot day^{-1}$. However, these authors also concluded that the positive outcomes were more consistent within an amateur population compared to well-

trained athletes. This finding contributed to the development of the experimental protocol by providing additional evidence regarding the optimal duration and dosage of omega-3 supplementation for well-trained endurance cyclists, specifically by administering a 1600 $mg \cdot day^{-1}$ dose of omega-3 for the total of 56-days (8-weeks).

2.7 Food diaries, Training logs, Athlete wellbeing and Illness Questionnaires

Food diaries are important for sports practitioners as they allow for a true representation of an athlete's dietary intake, avoiding a reliance on their perception (Burke, 2001). This precision is beneficial for cyclists, who need to ensure their nutrition supports optimal performance, injury prevention, and delays the onset of fatigue (Rothschild et al., 2020). A year-long study by Basiotis and colleagues (1987), found that ~41 days of a food intake record is required to estimate an individual's nutrient intake accurately. However, this varies depending on the individual and the nutrient being monitored. Furthermore, Magkos and Yannakoulia (2003), found that 1-day food diaries are not suitable for individual assessment due to significant intra-individual variability in daily food intake. Therefore, it is recommended to have a minimum of a 3-day record, which should include both weekdays and weekends, as this more accurately reflects an athlete's normal intake and reduces bias, which is also supported by others (Havemann & Goedecke, 2008; O'Keeffe et al., 1989; Viner et al., 2015) who have also used cyclists to asses' typical dietary practices (estimations of energy and macronutrient intakes). Collectively, these authors also conclude that a 7-day record provides the most reliable form of data collection.

Furthermore, Braakhuis and colleagues (2003), investigated self-reporting variability in 52 elite athletes (no descriptive data provided) by 53 different Australian sports dietitians. The key findings from this study were that the most significant errors in food diaries resulted from subjects not accurately representing their habitual dietary patterns. To minimise these errors, these researchers recommend that food diaries should be detailed and accurate, use clear and comprehensible language for the athlete, and to include longer durations to record nutrients that are not commonly found in most foods to account for fluctuations. Furthermore, weighing food items can significantly improve the accuracy of food diaries by allowing participants to record their eating habits just before consumption, and therefore can reduce omissions due to memory loss (Ortega et al., 2015). However, this method can increase subject burden and often can result in a decline in the quality of information as well as increasing the number of recording days (Bailey, 2021; Thompson & Byers, 1994). Even though longer duration (7 days) food diaries often provide more reliable data on athletes' food intake it is important that they balance accuracy with practicality and implementation.

Training logs are important for cyclists as they provide objective data pertaining to training volume and intensity, assisting both the athlete and coach(es) to balancing training loads and achieve performance goals (Meeusen et al., 2013). They are also beneficial for monitoring individual progress and making necessary adjustments to training variables, such as duration of sessions (Sanders et al., 2017). The main components of training include frequency, duration, and intensity (Jeukendrup & Diemen, 1998), and therefore for should be included within their training logs. Frequency and duration can be easily determined, however measuring intensity uses more specific metrics such as; speed, HR (average and maximal), power output, and RPE (Faria et al., 2005; Jeukendrup & Diemen, 1998).

Due to technological improvements, it is easier for cyclists to complete training logs at home, however, this has led to an increased uncertainty around the variability of the data and the response rates (Sanders et al., 2017). Therefore, properly calibrated devices are essential for accurate measurement of training intensities, and specialised training apps like Strava and TrainingPeaks can help monitor determinates (Camacho-Torregrosa et al., 2021). Tracking HR is important for determining if cyclists are training within their desired zones (Jeukendrup & Diemen, 1998), which can help the improvement of muscular flexibility strength, endurance, and coordination (Dong, 2016). Power is also important to monitor during cycling, as according to Vogt and colleagues (2006), it is the most direct measure of exercise intensity. However, power can be influenced by various outside factors, such as temperature (Atkinson et al., 2007). In an attempt to quantify this, Tatterson and colleagues (2000), investigated the impact of heat stress on 11 elite road cyclists (VO_2peak 4.9 ± 1.0 $L \cdot min^{-1}$). During this protocol, participants completed two 30-min TT's in an environmental chamber (set to either 32°C or 23°C, respectively). The authors found a significant reduction (↓ 6.5%; $p < 0.05$), in power output during the 32°C TT (323 ± 8 W), compared to the 23°C TT (345 ± 9 W).

As previously mentioned, an effective method for monitoring NMF is through self-reported questionnaires, especially as regular training and competitions can increase stress factors that influence an athlete's wellbeing (Clemente et al., 2019). Wellbeing is affected by numerous physical and psychological factors, which can be assessed by variables such as fatigue, sleep quality, general muscle soreness, stress levels, and mood (Haddad et al., 2013; Hooper & Mackinnon, 1995). Monitoring fatigue is important as it helps athletes ensure they are adapting to their training programmes (super compensation), while minimising the risk of

overreaching, injury, or illness (Halson, 2014). For cyclists, maintaining a good sleep quality and quantity is essential, as it has been positively linked to with performance measures such as strength and anaerobic power (Walsh et al., 2021). Additionally, monitoring muscle soreness helps athletes reduce the risk of injury or over-exertion (Montgomery & Hopkins, 2013). Tracking stress levels and mood is also an important variable(s) for ensuring optimal psychological well-being (Edwards, 2006).

These findings contributed to the design of the experimental protocol by expanding on existing research regarding the effectiveness of food diaries, training logs, and athlete well-being questionnaires in a sporting population – specifically well-trained endurance cyclists – through the supplementation of a 1600 mg omega-3 dose and a 20 g protein supplement beverage.

2.8 Thesis Aims

The primary aims of this thesis are to (1) systematically review and analyse previous research regarding whether omega-3 supplementation can affect a cyclists endurance performance, recovery and NMF, (2) investigate whether a 1600 $mg \cdot day^{-1}$ of omega-3 supplementation over an 8-week period can positively impact endurance performance in cyclists and to (3) determine whether this supplementation protocol can effectively reduce the presence of NMF following high-intensity endurance exercise. This research will also contribute to existing evidence regarding the optimal dosage and duration of supplementation for well-trained endurance cyclists.

The secondary aims of this thesis are to 1) provide further evidence towards the validity of TT's as a reliable method for assessing a cyclist's performance and 2) explore whether a CMJ protocol is a successful indicator of NMF in endurance cyclists. Furthermore, we hope this research will contribute to the body of knowledge quantifying the effectiveness of food diaries, training logs, and the use of an athlete wellbeing questionnaires in cyclists.

Chapter 3. Systematic Review

3.1 Method used for the Systematic Search

This systemic search aims to analyse previous research regarding whether omega-3 supplementation can affect a cyclists endurance performance, recovery and NMF. In total, this search found *n* = 11 papers which met the inclusion criteria.

3.1.1 Search Parameters

When developing a search strategy, the research question was first formulated through the utilisation of Population (P), Exposure (E), and Outcome investigated (O), PEO, which was adapted by (Bettany-Saltikov, 2016) Therefore, cyclists were the population investigated, the exposure was omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation, and the outcome investigated was endurance capacity, performance, and/or neuromuscular fatigue.

The Web of Science and EBSCO search engines were utilised to access the following databases: Web of Science Core Collection, BIOSIS Citation Index, BIOSIS Previews, KCI-Korean Journal Databases, Medline, SciELO Citation Index, and SportDiscus. All the studies utilised within this systematic review were derived from these databases and these search engines were used as they produce high-quality articles and are regarded as a trusted citation base for researchers (Liu, 2019). This search was carried out between 24/04/2023 to 05/05/2023 and to ensure accurate results, the use of synonyms, truncations, wildcards, and Boolean terms were included. The final search term included:

“omega-3" OR “n-3 fatty acid*” OR “polyunsaturated fatty acid*” OR “eicosapentaenoic acid*” OR “EPA” OR “docosahexenoic acid” OR “DHA”

AND

“endurance” OR “athlete” OR “endurance performance” OR “exercise recovery” OR “neuromuscular recovery”.

Results were limited to academic journals written in the English language; (journals previously translated into the English language were also accepted) and full text availability. Furthermore, search limiters were adapted to only return articles published from 2000 to the present day (05/05/2023) to ensure up-to-date research was reviewed.

3.1.2 Eligibility Criteria

Once the papers had been identified, their eligibility was assessed by a single reviewer. Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020, developed by (Page et al., 2021), was used to filter these articles, (see Figure 2). Prior to screening, all duplicates were removed by the reviewer. Next, the title and abstracts were screened, and any clearly irrelevant records were removed, this was followed by a full-text screening of the remaining articles. The reference lists of the eligible articles were also reviewed to identify any additional studies that may have been missed during the initial search. However, no further studies were deemed suitable for inclusion.

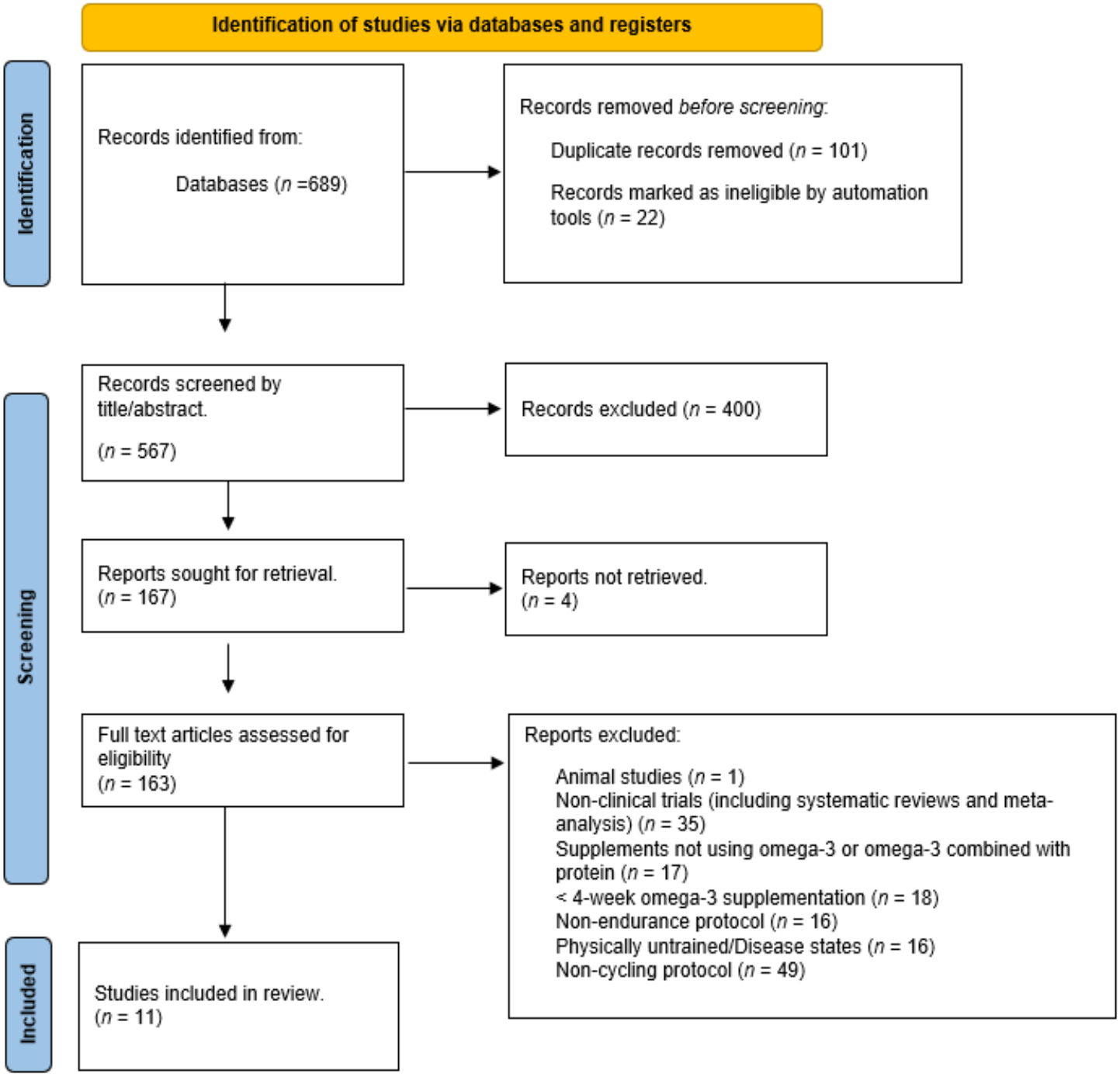

Figure 2. PRISMA 2020 flow chart for screening process

3.1.3 Screening Process

From this systematic search, a total of 689 papers were identified. Firstly, duplicate studies were removed using EndNote (Version X8, Thomson Reuters, Philadelphia, PA, USA). Following this, a total of 567 papers were then further screened against the eligibility criteria by the reviewer, with 11 studies included in the final analysis.

Eligibility was determined through the inclusion/exclusion criterion, as described in Table 1 (below). Endurance protocols were determined through protocol specifications, ether defined as eccentric (*n* = 15) or damaging (*n* = 1). The cycling protocols had to clearly specify the use of a bike and/or cycle ergometer as part of the research protocol to be accepted.

Table 1. Inclusion and exclusion criteria for systematic search papers

Inclusion Criteria	Exclusion Criteria
Published after 2000	Published before 2000
English language	Non-English language
Full text available	Full text not available
Human subjects only	No Animal models
Experimental research	Non-experimental research
Adults (18+)	Children (< 18)
Omega-3 supplements and omega-3 combined with protein supplements, regardless of delivery method or dose	Supplements not using omega-3 or omega-3 combined with protein
≥ 4-week omega-3 supplementation	< 4-week omega-3 supplementation
Endurance protocol	Non-endurance protocol
Cycling protocol	Non-cycling protocol
Well trained/ semi-professional/ athletes	Physically untrained / Disease States

3.1.4 Assessment of Quality

The quality of studies found during the screening process was assessed using the Physiotherapy Evidence database (PEDro scale; 1999) to score each article. This tool is designed to evaluate the methodological quality of trials and is widely used in evidence-based practice. The PEDro scale includes an 11-item checklist that yields a maximum score of 10 as

no points are rewarded for meeting the inclusionary criterion. The 11 statements include: (1) specification of eligible criteria, 2) random subject allocation into groups, with crossover studies being randomly allocated in order which treatments were received, 3) concealment of allocation, 4) group baselines are similar regarding the most important prognostic indicators, 5) all subjects were blinded, 6) therapists who administered the therapy were all blinded, 7) all assessors who measured at least one key outcome were all blinded, 8) measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to group, 9) subjects with outcome measures available, received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analysed by "intention to treat", 10) results of between-group statistical comparisons are reported for at least one key outcome, and 11) both point measures and measures of variability for at least one key outcome are provided.

This rating system was deemed suitable for this research as it has an ability to objectively assess the internal validity of studies (De Morton, 2009). Each paper was independently analysed by one researcher and then given a PEDro score. Any PEDro scores < 6 were deemed unacceptable, as according to the PEDro scale guidelines (PEDro scale, 1999), and hence these papers would not be utilised for analysis. As indicated in Table 2 below, all 11 papers met the requirements to be included in the analysis.

Table 2. PEDro score rating for the final 11 papers.

Study ID	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	PEDro Score
(Ávila-Gandía et al., 2020)	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	11
(Da Boit et al., 2015)	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	10
(De Salazar et al., 2020)	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	11
(Hingley et al., 2017)	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	10
(Lewis et al., 2015)	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	11
(López-Román et al., 2019)	+1	+1	0	+1	0	0	0	+1	+1	+1	+1	7
(Macartney et al., 2014)	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	11
(McAnulty et al., 2010)	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	10
(Nieman et al., 2009)	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	10
(Peoples et al., 2008)	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	10
(Poprzecki et al., 2009)	+1	+1	0	+1	0	0	0	+1	+1	+1	+1	7

3.2 Results found from the Systematic Search

3.2.1 Participant Characteristics

This systematic search identified a total of 11 papers that met the inclusion criteria. The total sample size from all studies combined was 337 participants, of which *n* = 269 were male (79.8%), and *n* = 20 were female (5.9%), and *n* = 48 were not reported (14.3%). Furthermore, the training experiences were varied as participants were either active *n* = 124 (36.7%), amateur at reginal level *n* = 43 (12.8%), trained *n* = 123 (36.5%), or well-trained n = 47 (13.9%), with this being determined through pre-screening questionnaires.

3.2.2 Source Matrix

After the final 11 papers had been screened for their quality, they were further analysed, and data extracted by the same researcher using a source matrix. Findings have been reported and discussed, with the study details of the research found in the 11 papers that meet the inclusion criteria, shown in Table 3 (below).

Table 3. Source matrix (design, sample size, supplementation ingestion strategy, method of study, and significant findings) of the final 11 papers

	Design	Sample Size	Ingestion Strategy	Method	Findings
(Ávila-Gandía et al., 2020)	Double blind, placebo controlled, randomised, balanced parallel study using a ramp cycling test to exhaustion to analyse aerobic metabolism.	38 male amateur cyclists (aged 18 or over) competing at a regional level. The absolute VO_2 scores for the placebo group were 2729 ± 340 $ml{\cdot}kg{\cdot}min^{-1}$ and 2792 ± 305 $ml{\cdot}kg{\cdot}min^{-1}$ for the experimental group.	Participants were randomly assigned into 2 groups: 1) Placebo (sunflower oil), *n* = 18. 2) Omega-3 (325 mg DHA [docosahexaenoic acid] & 40 mg EPA [eicosapentaenoic acid]), *n* = 20. Participants ingested 3 soft gels every morning before breakfast for 30-days.	Participants began by performing an initial incremental exercise test to exhaustion (starting at 50 W and increasing 5 W every 12 s with a cadence between 60 to 100 rpm (revolutions per minute). Exhaustion was achieved when cadence dropped below 20 rpm, or they volunteered to stop. Afterwards, a recovery stage began for 4-min and 30 s at 50 W. During this time, micro-capillary blood was collected (at the beginning of the recovery, 1-min and 30 s, 3-min & 4-min and 30 s). This was followed a 30-day supplementation period with omega-3 or placebo, after which participants repeated the same protocol.	Mean power output at ventilatory threshold 2 significantly improved (p = 0.006) after supplementation absolute (omega-3 vs placebo: 6.33 – 26.54 W; CI 95%) compared to placebo. Recovery HR significantly improved during the recovery phase in the omega-3 group compared to placebo (p = 0.005).
(Da Boit et al., 2015)	Double blind, randomised, parallel study to determine the immune function and performance	19 male and 18 female active cyclists (mean age was 25.8 years). The VO_2max scores were 41.6 ± 7.8 $ml{\cdot}kg{\cdot}min^{-1}$	Participants were randomly assigned into 2 supplement groups: 1) Placebo (unspecified oil), *n* = 19 (male) and *n* = 10 (female). 2) Omega-3 (60 mg EPA, 30 mg DHA & 61 ug	Firstly, a baseline blood sample was collected from the antecubital vein. Afterwards, they performed an initial incremental exercise test to exhaustion (increasing 30 W for males and 20 W for females every minute with a cadence between 70 to 90 rpm). This was followed by a cycling TT, set at the time to complete a specific amount of work at 70% Wmax, with ~80 rpm cadence.	The time to complete the TT in the omega-3 group significantly improved ($p < 0.05$) from pre- to post-omega-3 supplementation, 83.9 ± 14.7 to 85.4 ± 19.8 min. Whilst the TT times in the placebo group significantly improved ($p < 0.05$) from pre- to

	during fatigued cycling.	for the placebo group and 43.6 ± 6.4 $ml \cdot kg \cdot min^{-1}$ for the omega-3 group.	astaxanthin) sourced from krill oil, *n* = 18 (male) and *n* = 8 (female). Participants were instructed to consume the capsules (2 g) per day for 6-weeks.	Further blood samples were taken 1 h and 3 h post-exercise protocol. The TT and blood samples were repeated after a 6-week supplementation (krill oil or placebo) period.	post- supplementation, 80.5 ± 11.2 to 84.7 ± 17.1 min. The omega-3 index significantly increased ($p < 0.05$) from pre- to post-placebo supplementation, 5.20 ± 1.28 to 5.40 ± 1.19 AU. Furthermore, a significant increase ($p < 0.05$) in the omega-3 index was also found int he omega-3 group from pre- to post-supplementation, 5.32 ± 1.36 to 6.76 ± 1.66 AU.
(De Salazar et al., 2020)	Double blind, parallel, unicentric, controlled, and randomised experimental study to measure oxidative stress during moderately intense, long	56 physically active males (age of participants was not specified) completed the trial procedures. VO_2max scores for each group were 75.8 ± 9.3 $ml \cdot kg \cdot min^{-1}$ (group 1) 74.9 ±	Participants were randomised into 5 separate supplement groups: 1) Placebo (refined sunflower oil), *n* = 13. 2) Omega-3 (350 mg DHA), *n* = 10. 3) Omega-3 (1050 mg DHA), *n* = 10. 4) Omega-3 (1750 mg DHA), *n* =12.	Firstly, participants had to complete an incremental exercise test to exhaustion (12 $km \cdot h^{-1}$ with load increases of 2 $km \cdot h^{-1}$ every 1-min, maintaining a constant slope of 2% with a cadence between 60 to 100 rpm). Exhaustion had been reached when the participant had a cadence below 20 rpm. This was followed 1-week later by a square-wave endurance cycling test (constant load at the same speed at 75% VO_2max) for 90-min. Venous blood samples were taken from the antecubital vein 10-min before any physical exercise and urine sample were taken 90-min and	When comparing the pre- to post-supplementation results during the square-wave endurance cycling test, groups 1, 2, and 3 found a significant decrease in oxidative stress ($p < 0.01$), whilst groups 4 and 5 found no significant difference ($p > 0.05$). Omega-3 levels significantly increased ($p < 0.05$) in all groups from pre- to post-omega-3 supplementation

	duration cycling.	5.7 ml·kg· min^{-1} (group 2) 76.7 ± 5.9 ml·kg· min^{-1} (group 3) 76.3 ± 5.0 ml·kg· min^{-1} (group 4) 79.9 ± 12.7 ml·kg· min^{-1} (group 5), respectively.	5) Omega-3 (2450 mg DHA), *n* =10. Supplements were presented in identical soft gel packets and were told to ingest one packet every morning before their breakfast for 4-weeks.	24 h post exercise to measure oxidative stress. Afterwards, participants were randomly assigned to their supplement group and ingested their supplement for 4-weeks. After the supplementation period, they returned to repeat the square-wave endurance cycling test, blood and urine samples procedures.	(excluding the placebo group; $p >$ 0.05); group 2 was 5.9 ± 1.6 to 6.7 ± 1.6 AU, group 3 was 5.8 ± 1.8 to 6.6 ± 1.9 AU, group 4 was 5.4 ± 1.2 to 6.6 ± 1 AU, and group 5 was 6.2 ±1.5 to 7.7 ± 1.2 AU.
(Hingley et al., 2017)	Double-blind placebo-controlled (soy oil) study. Determined through TT times in a state of fatigue.	26 male, trained athletes (aged between 18 to 40 years), completing in ether cycling (*n* = 16) or running (*n* = 10). The predicted peak power aerobic power for the omega-3 group was 334 ± 30 W, 51.0 ± 8.7 ml·kg· min^{-1} and the	Participants were allocated into 2 separate supplementation groups: 1) Placebo (soy oil), *n* = 13 2) Omega-3 (140 mg EPA + 560 mg DHA) of tuna fish oil, *n* = 13. The supplements were presented in coded, sealed blister packs to achieve double blinding. Participants were instructed to consume 2 unmarked coated capsules per day for 8-weeks.	Visit 1: Familiarisation with the equipment and assessment methods. Visit 2: Participants began by completing 3 sets of isometric quadriceps strength testing to measure muscle voluntary contraction (MVC) by sitting upright at hip flexion at 90° for 5 s with a 60 s recovery. This was followed by a 10-min SSC at 125 W, and immediately afterwards participants completed a maximal cycling power (3 × 6 s with 60 s active recovery at 80 W; 70 to 80 rpm), with the highest power output being recorded. After this, they completed a Wingate fatigue protocol (6 × 30 s with 150 s active recovery at 80 W; 70 to 80 rpm) and repeated the maximal cycling power. Participants finished with a 5-min cycling TT and	The SSC found no significant differences ($p >$ 0.05) in VO_2 from pre- to post- supplementation in both the placebo (1.69 ± 0.03 to 1.71 ± 0.03 L·min^{-1}) and the omega-3 groups (1.65 ± 0.05 to 1.68 ± 0.02 L·min^{-1}). There were no significant differences *(p >* 0.05) found in power output during the maximal cycle outputs from pre- to post-supplementation in both the placebo (267 ± 19 to 267 ± 19 W) and omega-3 groups (253 ± 16 to 265 ± 16 W). Furthermore, no

		placebo were 338 ± 34 W, 51.5 ± 11.0 ml·kg·min^{-1}.		repeating the MVC. After this, participants were allocated into either the omega-3 or placebo groups where they were supplemented for an 8-week period. Visit 3: This was a repeat of visit 2 and was conducted after the 8-week supplementation period.	significant differences *(p* > 0.05) found in the MVC from pre- to post- supplementation in the placebo (273 ± 19 to 251 ± 19 Nm) and omega-3 groups (287 ± 17 to 283 ± 16 Nm). However, there was a significant decrease (*p* < 0.05) in relative oxygen consumption during the TT in the omega-3 group from pre- to post-omega-3 supplementation, no data provided to -154 ± 59 ml·O^{2}·min· 100 W).
(Lewis et al., 2015)	Randomised, placebo-controlled, parallel study (interspaced by 21 days), to determine changes in neuromuscular function, performance,	30 male, well-trained athletes (aged between 20 to 30 years), who all competed in summer Olympic sports. Their VO_2max measures were 49.3 ± 14.2 ml·kg·	Participants were randomly allocated into 2 supplementation groups: 1) Placebo (olive oil & 1000 IU vitamin D), *n* = 12. 2) Omega-3 (375 mg EPA, 230 mg DPA & 510 mg DHA) of seal oil, *n* = 18. Participants were instructed to orally take 2 to 2.5 ml twice a day for 21	Familiarisation: VO_2max test (resistance set at 50 W, 100 W & 150 W for 2- min each then increasing 25 W per minute) was determined as the highest value achieved over a 20 s period. Visit 1: Venous blood samples (8 ml from the antecubital vein) and electromyography (EMG) were taken at the beginning of visit 1 & 2. Neuromuscular testing included 3 maximal squat jumps and CMJ on a force plate, then performing as many push ups as they could in 1-min. After a 5-min rest, participants performed 4 to 6 warm up	In the omega-3 group, there was an increase (no *p* value provided) in MVC force from pre- to post-omega-3 supplementation, 643 ± 144 to 670 ± 175 N; 4.1%. However, the placebo group found no significant differences (no *p* value provided) from pre- to post- supplementation, 677 ± 107 to 683 ± 154 N; 0.03 %. Furthermore, both the omega-3

	and fatigue via VO_2max, neuromuscular testing, Wingate testing, and TT.	min^{-1} in the omega-3 group and 48.9 ± 3.4 $ml \cdot kg \cdot min^{-1}$ in the placebo group.	days. Furthermore, they had to let the oil remain in their mouth for 1-min before swallowing.	reps at 20%, 40%, 60% or 80% of their 10-rep max before performing the maximum number of reps at 100% 10 RM max squat weight. This was followed by a 30 s Wingate test at 7.5% of bodyweight. Finally, participants performed a 250 kJ cycling TT with resistance set at 75% VO_2max. After 21 days of supplementation (omega-3 or placebo), participants came in for visit 2 which was a repeat of visit 1.	and placebo groups found an unclear inference ($p > 0.05$) from pre- to post- supplementation in the squat jumps, CMJ, push ups, back squats, and TT tests. The omega-3 group found a likely beneficial decrease in fatigue from pre- to post- omega-3 supplementation, 54.8 ± 9.4 to 54.6 ± 10.3% drop**.** However, the placebo group found a significant increase ($p < 0.05$) in fatigue from pre- to post- supplementation, 49.1 ± 5.8 to 53 ± 5.5% drop.
(López-Román et al., 2019)	Single-centred open label study to determine the difference in aerobic conditions in competitive cyclist's vs leisure cyclists.	13 male amateur (n = 7) and competitive (n = 6), aged 18 and over. VO_2max measures were 61.7 ± 7 $ml \cdot kg \cdot min^{-1}$ in the competitive group and 44.8 ±	Participants were asked to ingest 2.1 g omega-3 supplement (3500 mg DHA) called Algatrium every day for 3-months. As every participant received the same supplement, they were presented with an open label.	Participants performed a maximal incremental cycling endurance test, cycling with a continuous increase of 25 W every minute, starting from 70 W, until exhaustion. Participants had to maintain a cadence between 60 to 100. Afterwards, participants had a 3-month DHA supplementation period, after which they returned to complete the same procedure.	Participant VO_2max results showed no significant differences from pre- to post- omega-3 supplementation in the competitive cyclists, 61.7 to 59.5 $ml \cdot kg \cdot min^{-1}$ ($p = 0.18$) and the non-competitive cyclists, 44.8 to 46.5 $ml \cdot kg \cdot min^{-1}$ ($p = 0.23$), respectively. The p value

		1.6 ml·kg· min^{-1} in the non-competitive group.			between competitive groups was 0.1.
(Macartney et al., 2014)	Double blind, parallel study to determine if omega-3 supplementation could improve cardiovascular function (e.g. HR) at rest, during intense physical exercise, and during recovery.	26 physically fit males aged between 18 to 40 years old. The VO_2 peak data was 51 ml·kg· min^{-1} in the omega-3 group, and 51.5 ml·kg· min^{-1} in the placebo group.	Subjects were randomly assigned into 2 groups: 1) Placebo (soybean oil), *n* = 13. 2) Omega-3 (560 mg DHA & 140 mg EPA) from tuna fish oil, *n* = 13. Capsules were presented unmarked in blister packets and ingested 2 g per day for 8-weeks.	Subjects started by conducting at home resting HR and blood pressure readings by resting in bed for 20-min. A venous blood sample was taken before the cycling protocol. Starting with a 10-min steady cycle (125 W; cadence at 60 rpm), followed by Wingate sprints (6 x 30 s with 150 s recovery at 80 W). This was followed by a 5-min active recover at 80 W, then a 5-min work capacity trial. After which subjects were randomly allocated into ether the placebo or tuna fish oil supplementation group, which lasted for 8-weeks. After this, the participants repeated the same protocols.	There were no significant differences ($p > 0.05$) found in the participants resting HR and from both the omega-3 supplementation (58 [SEM 2] to 59 [SEM 3] beats·min^{-1}) and placebo (55 [SEM 3] to 57 [SEM 3] b·min^{-1}) groups**.** A significant decrease ($p < 0.05$) in HR during the SSC in the omega-3 (-22 [SEM 6] b·min^{-1}) comparison to the placebo group *(*$p > 0.05$; +1 [SEM 4] b·min^{-1}). Furthermore, there were no significant differences ($p > 0.05$) in the participants peak HR during the Wingate sprints in both the omega-3 (176 [SEM 1] b·min^{-1}) and placebo groups (174 [SEM 1] b·min^{-1}).

(McAnulty et al., 2010)	Randomised, crossover study to examine how omega-3 supplementation influences direct markers of oxidative damage after exhaustive exercise.	48 trained cyclists, aged between 20 to 30 years. The participants VO_{2peak} was 58.8 ± 31 ml·kg· min^{-1} in the placebo group, 66.6 ± 3.1 ml·kg· min^{-1} in the vitamin group, 62.9 ± 3.8 ml·kg· min^{-1} in the omega-3 group, and 59.8 ± 7.3 ml·kg· min^{-1} in the vitamin & omega-3 group.	Participants were randomly allocated into 4 supplementation groups: 1) Placebo (unspecified), *n* = 12. 2) Vitamin (2000 mg vitamin C, 800 IU vitamin E, 3000 IU vitamin A & 200 µg selenium), *n* = 12. 3) Omega-3 (2000 mg EPA and 400 mg DHA), *n* = 11. 4) Omega-3 and vitamin (a combination of groups 2 & 3), *n* = 13. Participants assigned into the placebo and omega-3 supplements were told to ingest 4 soft gel capsules per day (2 between 7:00 – 8:00 am on an empty stomach & 2 between 6:00 - 8:00 pm). However, subjects on the vitamin supplements took 8 (4	Participants were randomised into 4 supplementation groups (placebo, vitamin, omega-3, and omega-3 and vitamin). Participants completed a VO_2max test (25 W increase every 2-min starting from 150 W). Furthermore, participants had to complete a 3 h SSC at 57% Wmax (determined during their VO_2max test). Antecubital blood samples were collected before supplementation (between 7:30 and 9:00 am and after an overnight fast), before exercise, and 15-min post-exercise. The blood markers included F2-isoprostanes, plasma EPA & DHA, ferric-reducing antioxidant potential, and oxygen radical absorption capacity. The SSC and bloods were repeated post supplementation phase, which was then repeated after 6-week supplementation period.	These authors averaged their performance characteristics (e.g. HR, power, VO_2) during the 2 visits, finding no significant differences ($p > 0.05$) from pre- to post-supplementation in all groups. Furthermore, the omega-3 group found a significant increase ($p = 0.01$) in F_2-isoprostane from pre- to post-supplementation in comparison to the placebo and other vitamin groups which found no significant difference ($p > 0.05$).

			morning & 4 night) capsules a day at the same times and the omega-3 & placebo groups.		
(Nieman et al., 2009)	Randomised, double blinded, placebo-controlled study to measure exercise performance, inflammation and immune measures during intense exercise.	23 trained cyclists from local and college cycling clubs (aged not specified). The VO_2max scores were 66.3 ± 3.0 $ml \cdot kg \cdot min^{-1}$ in the placebo group, and 64.2 ± 3.2 $ml \cdot kg \cdot min^{-1}$ in the omega-3 group.	Participants were randomised into 2 different supplementation groups: 1) Placebo (soybean oil), *n* = 12. 2) Omega-3 (2,000 mg EPA & 400mg DHA), *n* = 12. The supplementation period lasted for a 6-week period, with both sets of capsules looking identical in appearance. Participants were told to ingest 2 capsules on an empty stomach (between 7 to 8 am) and 2 before their evening meal (between 6 to 8 pm).	Participants started by completing a VO_2max test (25 W increase every 2-min, starting at 150 W). After this, participants cycled for 3 h 57% W_{max}, with 10-km TT inserted during the final 15-min of each 3 h bout. Venous blood and saliva samples were measured for c-reactive protein and creatine kinase. They were collected immediately after the 3 h exercise bout and 14 h postexercise. Participants were then randomly assigned into the placebo or omega-3 supplementation group, for a 6-week period. Afterwards, the participants returned to repeat the same exercise protocol.	This study averaged their performance characteristics (e.g. TT times, power, HR, and VO_2), finding no significant differences from pre- to post-supplementation ($p > 0.05$). Furthermore, no significant differences ($p > 0.05$) were found in both the placebo and omega-3 groups blood and saliva analysis (c-reactive protein and creatine kinase).

(Peoples et al., 2008)	Double blind, parallel study to examine the effects of fatigue oxygen consumption during exercise.	16 male well-trained cyclists (aged between 20 to 30 years). There $VO_{2\ peak}$ was 66.8 ± 2.4 $ml{\cdot}kg{\cdot}\ min^{-1}$ in the placebo group and 6.8 ± 1.4 $ml{\cdot}kg{\cdot}\ min^{-1}$ in the omega-3 group.	Subjects were randomly allocated into 2 supplement groups: 1) Placebo (olive oil), *n* = 7. 2) Omega-3 (800 mg of EPA & 2400 mg of DHA) sourced from tuna fish oil, *n* = 9. The supplementation period lasted for 8 weeks with 8 capsules per day.	The participants first started by performing a peak oxygen consumption test. Starting with a 10-min warm up at 150 W, after which 2 W increased every 3 s until the participant could not maintain a cadence greater or equal to 40 rpm. This was followed by a sustained submaximal cycling test to exhaustion one week later, set at 55% peak workload from the oxygen consumption test. Participants were told to cycle until voluntary exhaustion with data being collected within the first 60-min and during the final min before exhaustion. Venous blood samples were taken before and after the supplementation period to measure omega-3 levels in the red blood cell membranes. Participants then repeated this exercise protocol after their supplementation period.	There were no significant differences ($p > 0.05$) in VO_{2peak} from pre- to post-supplementation in the placebo group (66.8 ± 2.4 to 67.2 ± 2.3 $ml{\cdot}kg{\cdot}\ min^{-1}$) and in the omega-3 group (68.3 ± 1.4 to 67.2 ± 1.2 $ml{\cdot}kg{\cdot}\ min^{-1}$). Furthermore, there were no significant differences ($p > 0.05$) in the mean respiratory exchange ration during the steady cycle from pre- to post-supplementation in the placebo group (0.9 ± 0.02 to 0.9 ± 0.03 O_2) and the omega-3 group (0.9 ± 0.02 to 0.9 ± 0.03 O_2).
(Poprzecki et al., 2009)	Randomised study to determine the modification of blood antioxidant levels during	24 healthy and moderately trained male students (no specific age provided). Their VO_2max scores	Subjects were randomly assigned into 2 separate supplement groups: 1) Placebo (gelatine), *n* = 12.	Participants started by cycling maximal power output test (Wmax) by cycling at 100 W for 5-min and increasing by 50 W every 2.5-min until HR reached 160 ($b{\cdot}min^{-1}$) This was followed by a 1 h cycle ergometer test at 60% Wmax. Participants cycled at a pedalling rate of 60 rpm for the first 45-min, increasing to the maximal rate for the	There were no significant differences ($p > 0.05$) in the omega-3 and placebo groups performance variables (HR, VO_2, work output, and respiratory exchange ration) during the 1 h cycle from pre- to post-

	fatigued endurance exercise.	were 50.4 ± 8.9 $ml \cdot kg \cdot min^{-1}$ in the placebo group, and 53.2 ± 11.4 $ml \cdot kg \cdot min^{-1}$ for the omega-3 group.	2) Omega-3 (1300 mg; 30% EPA, 20% DHA & 4 mg x-tocopherol), *n* = 12. The supplementation period lasted for 6-weeks.	final 15-min. Antecubital venous blood samples were taken at 3 timepoints: before exercise, after exercise cessation, and after 1 h of recovery. This protocol was repeated after a 6-week omega-3 or placebo supplementation period.	supplementation. The omega-3 group found a significant increase ($p < 0.05$) in uric levels 1 h post exercise from pre- to post-supplementation, 4.7 ± 0.6 to 5 ± 0.6 UA. However, the placebo group found a significant decrease ($p > 0.05$) in uric levels 1 h post exercise from pre- to post-supplementation, 5.5 ± 0.7 to 5.1 ± 0.6 UA.

CMJ = Countermovement jump, DHA = Docosahexaenoic acid, DPA = Docosapentaenoic acid, EMG = Electromyography, EPA = Eicosapentaenoic acid, g = grams, HR = Heart-rate, h = hours, kg = kilograms, kJ = kilojoules, mg = milligrams, MVC = Maximal voluntary contraction, RPM = Revolutions per minute, s = seconds, SSC = Steady state cycle, TT = Time trial, W = Watts, Wmax = Maximal work capacity.

This search identified a total of *n* = 11 papers which met the inclusion criteria. Below, outlines and compares the different methods utilised by these authors (Ávila-Gandía et al., 2020; Da Boit et al., De Salazar et al., Hingley et al., 2017; Lewis et al., 2015; López-Román et al., 2019; Macartney et al., 2014; McAnulty et al., 2010; Nieman et al., 2009; Peoples 2008; Poprzecki et al., 2009)**,** including the dosages and durations of the omega-3 supplementation. Additionally, these protocols focused on measuring endurance, causing fatigue, and measuring NMF within a cycling population.

3.2.3 Methods of Omega-3 Supplementation

Out of the 337 participants, *n* = 179 received an omega-3 supplementation (53.1 %) and *n* = 159 received a placebo (47.9 %). The supplementation periods ranged between 21 days to 56 days (46 average days), with dosages of omega-3 ranging from 350 mg to 3200 mg per day (average was 1595.4 mg per day). The placebos included sunflower oil (Ávila-Gandía et al., 2020; De Salazar et al., 2020), soy oil (Hingley et al., 2017), olive oil (Lewis et al., 2015; Peoples et al., 2008), soya bean oil (Macartney et al., 2014; Nieman et al., 2009), multivitamin (McAnulty et al., 2010), gelatine (Poprzecki et al., 2009), or unspecified (Da Boit et al., 2015). The ingestion methods used throughout these studies included, soft gels (Ávila-Gandía et al., 2020; De Salazar et al., 2020; McAnulty et al., 2010; Nieman et al., 2009), capsules (Da Boit et al., 2015; Hingley et al., 2017; Macartney et al., 2014; Peoples et al., 2008), oil (Lewis et al., 2015; López-Román et al., 2019), or unspecified (Poprzecki et al., 2009). Also, some studies used a specific brand of omega-3 supplements such as Algatrium (López-Román et al., 2019), Nu-Mega (Hingley et al., 2017; Macartney et al., 2014; Peoples et al., 2008), Auum Inc.,

Timmons, On (Lewis et al., 2015), Rybasol Pronova Biocare (Poprzecki et al., 2009), and Tridocosahexaenoine-AOX® (Ávila-Gandía et al., 2020; De Salazar et al., 2020).

Only *n* = 4 studies gave specific instructions of when to ingest the supplements. Ávila-Gandía and colleagues (2020) recommended three soft gels every morning with their breakfast, De Salazar and colleagues (2020), recommended 1 soft gel every day before breakfast on an empty stomach, and McAnulty and colleagues (2010) and Nieman and colleagues (2009), recommended two in the morning (between 7 to 8 am), on an empty stomach and two before their evening meal (between 6 to 8 pm).

To ensure participants were adhering to their supplements, Ávila-Gandía and colleagues (2020) gave verbal reminders as well as asking their participants to return empty supplement packets. Hingley and colleagues (2017) performed a capsule count and monitored omega-3 changes in participant erythrocyte membranes, which followed the protocol of an earlier study (Macartney et al., 2014). McAnulty and colleagues (2010), contacted their participants weekly (via email) and instructed participants to bring back empty supplement boxes.

3.2.4 Protocols used to Measure Endurance

A total of *n* = 9 studies utilised a VO_2max protocol within their research design (Ávila-Gandía et al., 2020; Da Boit et al., De Salazar et al., Lewis et al., 2015; López-Román et al., 2019; McAnulty et al., 2010; Nieman et al., 2009; Peoples 2008; Poprzecki et al., 2009). However, *n* = 6 studies, only implemented their protocols to determine participant training status and/or to use the results as markers for future tests within their studies (Da Boit et al.,

2015; De Salazar et al., 2020; Lewis et al., 2015; McAnulty et al., 2010; Nieman et al., 2009). Therefore, only *n* = 4 studies compared participant VO_2max data from pre- to post-supplementation (Ávila-Gandía et al., 2020; López-Román et al., 2019; Peoples 2008; Poprzecki et al., 2009).

The starting resistance that was implemented were 50 W (Ávila-Gandía et al., 2020), 70 W (López-Román et al., 2019), 100 W (Poprzecki et al., 2009), 150 W (McAnulty et al., 2010; Nieman et al., 2009; Peoples 2008), 12 $km \cdot h^{-1}$ (De Salazar et al., 2020), self-selected cadence (Da Boit et al., 2015), or starting at 50 W, 100 W, and 150 W every 2-min (Lewis et al., 2015). With the W increasing by 5 W every 12 s (Ávila-Gandía et al., 2020), 25 W every 3 s (Peoples 2008), 2 km every 1-min (De Salazar et al., 2020), 25 W every 1-min (Lewis et al., 2015; Lewis et al., 2019), 25 W every 2-min (McAnulty et al., 2010; Nieman et al., 2009), and 25 W every 2.5-min (Poprzecki et al., 2009). However, Da Boit and colleagues (2015), set a different W increase (every min) depending on gender; 30 W for males and 20 W for females.

A total of *n* = 6, different tests were used to measure participant endurance. These included Wingate (Hingley et al., 2017; Macartney et al., 2014), maximal cycling power test (Hingley et al., 2017), work capacity trial (Maccartney et al., 2014), square-wave endurance exercise test (SWEET; De Salazar 2020), TT (Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; Nieman 2009), and SSC (Hingley et al., 2017; Macartney et al., 2014; McAnulty et al., 2010; Nieman et al 2009; Peoples 2008; Poprzecki 2009).

Both the studies who implemented the Wingate protocol used the same method; six sets of 30 s sprints with a 150 s active recovery at 80 W (Hingley et al., 2017; Macartney et al., 2014). Furthermore, Hingley and colleagues (2017), were the only authors to conduct a maximal cycling power test of 6 sets of 30 s sprints with a 50 s recovery phase. De Salazar and

colleagues (2020), were the only study to conduct a SWEET protocol, whereby participants were required to cycle at 75% VO_2max for 90-min. The different times set during the TT were 5-min (Hingley et al., 2017), 10-min immediately at the end of a 1 h SSC (Nieman 2009), and 20-min at 250 KJ (Lewis 2015). Alternatively, Da Boit and colleagues (2015) instructed their participants to cycle until they reached 70 % Wmax.

However, the most common endurance test utilised by these authors (Hingley et al., 2017; Macartney et al., 2014; McAnulty et al., 2010; Nieman 2009; Peoples 2008; Poprzecki 2009) was SSC, with durations ranging between 10-min (Hingley et al., 2017; Macartney et al., 2014), 60-min (Poprzecki 2009), and 3 h, respectively (McAnulty et al., 2010; Nieman 2009). Furthermore, Peoples and colleagues (2008) instructed their participants to cycle to exhaustion, however, only the first 60-min of data was recorded. Participants were instructed to cycle at 125 W (Hingley et al., 2017; Macartney et al., 2014), 57% Wmax (McAnulty et al., 2010; Nieman 2009), 55% peak workload (Peoples 2008), and 60% Wmax (Poprzecki 2009).

3.2.5 Protocols used to confirm the presence of Fatigue and to measure Neuromuscular Fatigue (NMF)

A total of *n* = 9, different methods were utilised to determine participants NMF; this included maximal voluntary contractions (MVC; Hingley et al 2017; Lewis et al., 2015), electrical stimulations (Lewis et al., 2015), maximal back squat jumps (Lewis et al., 2015), CMJ (Lewis et al., 2015), push ups (Lewis et al., 2015). Furthermore, to confirm the presence of fatigue some authors collected urine (De Salazar et al 2020), saliva (Nieman et al., 2009), and blood (Ávila-Gandía et al., 2020; Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015;

Macartney et al., 2014; McAnulty et al., 2010; Nieman et al., 2009; Peoples et al., 2008; Poprzecki 2009), samples during their exercise protocols.

Both MVC studies (Hingley et al., 2017; Lewis et al., 2015), measured their participants quadricep strength, however, Hingley and colleagues (2017), had their participants perform a total of three contractions for 5 s with a 60 s recovery in an unfatigued state and 15 - min post the Wingate test. Whilst Lewis and colleagues (2015) performed a total of five contractions for 5 s with a 60 s recovery, before and after their exercise protocol. Lewis and colleagues (2015) were the only authors to conduct *n* = 4, specific NMF tests (electrical stimulations, back squats, CMJ, and push ups). Electrical stimulations were conducted at 400 V, with stimulation increasing by 5 mA until the quadricep twitch force was not altered. Participants also had to complete four to six sets of maximal back squat jumps at 20%, 40%, 60%, and 80% of their 10-rep max (weights determined through participant training weights), followed by the maximal number of reps at 100 %. Furthermore, participants had to complete three sets of CMJ (timepoints not specified) and two sets of as many push ups as possible in 1-min, with 1-min rest.

To confirm the presence of fatigue, through oxidative stress, De Salazar and colleagues (2020) had their participants provide a 15 ml urine samples 24 h before and the day of their exercise protocols both pre- and post- supplementation. Furthermore, Nieman and colleagues (2009), collected saliva samples from their participants after an overnight fast, immediately post exercise, and 14 h post exercise both pre- and post-supplementation.

A total of *n* = 9, studies collected blood samples from their participants (Ávila-Gandía et al., 2020; Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; Macartney et al., 2014; McAnulty et al., 2010; Nieman et al., 2009; Peoples et al., 2008; Poprzecki 2009). These blood

samples were received from the antecubital/median cubital vein (Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; Macartney et al., 2014; McAnulty et al., 2010; Nieman et al., 2009; Peoples et al., 2008; Poprzecki 2009), or capillary (Ávila-Gandía et al., 2020). The amounts of blood taken from the participants at each timepoint were 125 μL (Ávila-Gandía et al., 2020), 4 ml (Da Boit et al., 2015), 8 ml (Lewis et al., 2015), 9 ml (Macartney et al., 2014), 10 ml (Peoples et al., 2008), or the authors did not specify (Hingley et al., 2017; McAnulty et al., 2010; Nieman et al., 2009; Poprzecki 2009). Only two studies provided specific details of when the bloods were taken. McAnulty and colleagues (2010) were between 7:30 – 9:00 am, following an overnight fast, whereas Nieman and colleagues (2009) collected their sample at 8:00 am (again, after an overnight fast).

Some studies only collected blood sample pre- exercise (Hingley et al., 2017; Lewis et al., 2015; Macartney et al., 2014; Peoples et al., 2008). However, some studies also collected blood samples post- exercise (Ávila-Gandía et al., 2020; Da Boit et al., 2015; McAnulty et al., 2010; Nieman et al., 2009; Poprzecki 2009). These timepoints included at the start of recovery (Ávila-Gandía et al., 2020; Da Boit et al., 2015; Nieman et al., 2009; Poprzecki 2009), 15 - min post- exercise (McAnulty et al., 2010), 1 h post- exercise (Da Boit et al., 2015; Poprzecki 2009), 3 h post- exercise, respectively (Da Boit et al., 2015), and 14 h post- exercise (Nieman et al., 2009). These blood samples were analysed for erythrocyte fatty acid composition (Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; Macartney et al., 2014; Peoples et al., 2008), plasma EPA and DHA (McAnulty et al., 2010; Nieman et al., 2009), oxygen radical absorption capacity (ORAC; McAnulty et al., 2010), ferric-reducing antioxidant potential (McAnulty et al., 2010), lactate (Ávila-Gandía et al., 2020), F_2-isoprostanes (McAnulty et al., 2010), natural killer cell cytotoxic activity (Da Boit et al., 2015), peripheral blood mononuclear cell (Da Boit

et al., 2015), thiobarbituric acid reactive substances (Da Boit et al., 2015), CK (Poprzecki 2009), uric acid, (Poprzecki 2009), plasma IL- 1ra (Nieman et al., 2009), plasma IL- 6 (Da Boit et al., 2015; Nieman et al., 2009), and plasma IL-8 (Nieman et al., 2009).

3.2.6 Secondary Testing

A total of *n* = 4 studies (Ávila-Gandía et al., 2020; Da Boit et al., 2015; De Salazar et al., 2020; Poprzecki et al., 2009), conducted a nutritional analysis during their supplementation period. Ávila-Gandía and colleagues (2020) instructed their participants to complete an initial prospective 24 h dietary recall before any exercise testing, followed by a 7-day food record to determine dietary intake. This process was completed post supplementation. Da Boit and colleagues (2015) instructed their participants to record their dietary intake prior to their pre-supplementation exercise protocols so that the same intakes could be repeated for post-supplementation. De Salazar and colleagues (2020) gave their participants qualitive and quantitative questionnaires which were analysed by a nutritionist to determine their dietary intake. Furthermore, Poprzecki and colleagues (2009), instructed their participants to register their meals three days prior to the first exercise protocol both pre- and post- supplementation to determine dietary intake.

3.3 Discussion of the Search Findings

This systematic search identified a total of 11 papers, of which all 11 met the inclusion criteria (see Table 1). The total sample size from all the found studies was 337 participants. The dosages of omega-3 ranged between 350 mg to 3200 mg per day (average was 1595.4

mg per day), with the different ingestion strategies including, soft gels, capsules, oil, or not specifying. Furthermore, the different methods used to measure endurance included, VO2max, Wingate, maximal cycling power test, work capacity trials, SWEET, and TT. Additionally, the protocols used to measure NMF included, MVC, electrical stimulations, maximal back squat jumps, CMJ, and push ups, with urine, saliva and blood samples used to confirm the presence of fatigue.

3.3.1 Omega-3 Supplementation

As previously mentioned in Chapter 3.2.3, the supplementation periods ranged between 21 to 56 days (46 average days). Lewis and colleagues (2015), acknowledged their short supplementation period of 21 days, with the justification that just seven days of omega-3 supplementation can lead to a sufficient increase in plasma EPA concentrations in 20 inactive adults (Metherel et al., 2009), and reduce muscle soreness in healthy adults (*n* = 11) performing eccentric bicep strength exercises (Jouris et al., 2011). However, as these studies did not include a cycling population or cycling protocols, they were not included within the search results. The average supplementation period was 46 days, however, the authors of these studies failed to justify the reason for the longer supplementation periods. Nieman and colleagues (2009) did acknowledge the limited research assessing the influence of omega-3 in an exercise context with a human populace, therefore past results are varied and inconsistent. This highlights the difficulty in determining a suitable duration of omega-3 supplementation to benefit an endurance cyclist's performance.

Furthermore, 53.1% of participants received an omega-3 supplementation while 47.9% received a placebo (*n* = 5 different placebos used). The benefit of placebo-controlled groups

is that it demonstrates the effectives of omega-3 interventions, as it helps minimise bias and enhances scientific reliability (Krol et al., 2020). However, not all study protocols can justify the use of placebos and therefore can gain scientific reliability through participant random variation (e.g. standard variation), retest correlation (e.g. estimation of magnitude of individual differences to a treatment), and systematic change in the mean (Hopkins, 2000).

The dosages of omega-3 ranged between 350 mg to 3200 mg per day (average was 1595.4 mg per day). Hingley and colleagues (2017), justified their lower dosage of 700 mg per day, based upon previous research suggesting this to be the threshold shown to maximise membrane incorporation in animals (Slee et al., 2010), increasing the omega-3 index (Macartney et al., 2014), elevating the skeletal muscle membrane (Anderson et al., 2002), and being linked to the total muscle fat in men (McGlory et al., 2014). Macartney and colleagues (2014), were the only study included within the final paper due to it including both a human populace and a cycling protocol. However, Poprzecki and colleagues (2009), who provided their cyclists with 1300 mg of omega-3 per day, stated that low dosages are still under debate as to whether they produce any beneficial effects in a human populace. Furthermore, Macartney and colleagues (2014) acknowledged that previous research (Buckley et al., 2009) measured the cardiovascular function (whilst training) in Australian footballers *(n* = 25*),* finding that higher-dosages of omega-3 (1920 mg per day for 5 weeks) could lower HR ($b \cdot min^{-1}$) during sustained endurance exercise. As this used a football populace with no cycling protocol, it was therefore not included within the final paper search. Also, De Salazar and colleagues (2020), investigated the effects of varying omega-3 dosages (350 mg, 1050 mg, 1750 mg, and 2450 mg per day for 4 weeks) on moderate-intensity long-duration aerobic exercise in amateur cyclists, finding that 1050 mg per day was the lowest dose to produce

positive results, with a trend towards neutralisation at the highest dosages of 2450 mg per day. These variable findings reflect the ongoing challenge in identifying the optimal omega-3 supplementation duration and dosage for enhancing cycling performance, especially when considering other variables such as training, diet quality, training load alongside other metabolic factors.

De Salazar et al., (202), recommend that future studies consider the total cumulative dose of omega-3 consumed, the standardisation of the supplement, the chemical structure and configuration of the omega-3 product, and the duration of treatment. Additionally, they recommended that the minimum effective dosage of omega-3 dosage should be ~1.05 g per day. Furthermore, there were no reports of adverse effects related to omega-3 supplementation in these aforementioned studies. However, it is unclear whether these authors provided their participants an opportunity to report such issues. Therefore, future studies should encourage participants to provide opportunities to report any side effects or issue they may experience during supplementation.

3.3.2 Omega-3 Ingestion Strategies

The ingestion methods included, soft gels (Ávila-Gandía et al., 2020; De Salazar et al., 2020; McAnulty et al., 2010; Nieman et al., 2009), capsules (Da Boit et al., 2015; Hingley et al., 2017; Macartney et al., 2014; Peoples et al., 2008), oil (Lewis et al., 2015; López-Román et al., 2019), or did not specify (Poprzecki et al., 2009). Only Lewis and colleagues (2015) provided justification for their choice of using oil, suggesting that oils might promote a more rapid

digestion of omega-3, potentially increasing total bioavailability and enhancing oxidation (Christensen et al., 1995; Paltauf et al., 1974; Tartibian et al., 2009).

The specific brands of omega-3 supplements were, Algatrium (López-Román et al., 2019), Nu-Mega (Hingley et al., 2017; Macartney et al., 2014; Peoples et al., 2008), Auum Inc., Timmons, On (Lewis et al., 2015), Rybasol Pronova Biocare (Poprzecki et al., 2009), and Tridocosahexaenoine-AOX® (De Salazar et al., 2020; Ávila-Gandía et al., 2020). Ávila-Gandía and colleagues (2020) acknowledge this choice was made due to the naturally high DHA levels. It is important for authors to provide a rationale for specific supplement choices, so readers do not interpret their results to be biased, even when stating that conflicts of interest have been avoided (Pannucci & Wilkins, 2010).

A total of *n* = 4 studies provided specific instructions of when participants should consume the omega-3 supplements (Ávila-Gandía et al., 2020; De Salazar et al., 2020; McAnulty et al., 2010; Nieman et al., 2009). However, these studies did not provide any justification for the specific timings of supplementation. To date, there is no conclusive evidence indicating the optimal time of ingestion for omega-3 supplements to maximise their efficacy.

Some authors adapted their protocols to monitor participant adherence throughout the supplementation period (Ávila-Gandía et al., 2020; Hingley et al., 2017; Macartney et al., 2014; McAnulty et al., 2014). Adherence to supplementation protocols are important to ensure that any potential omega-3 benefits can take place (Petróczi & Naughton, 2007). Phillips and colleagues (2021), suggest that adherence to medications (which could include dietary supplements) may be greater in the morning compared to the evening. However, further research is needed to evaluate the validity of behavioural timing consistency.

Malinowski and colleagues (2019), investigated the effect of fish oil administration methods on the tolerability and adherence in healthy adults *(n* = 60). These participants were separated into four ingestion methods; 1) with no food, *n* = 17, 2) with food, *n* = 12, 3) with only milk, *n* = 15, and 4) kept in the freezer, *n* = 11. Participants were instructed to consume two capsules, three times per day. Overall, the mean adherence rates were 68 % with no food, 65% with food, 78% with milk, and 63% from the freezer. These authors suggested that the high pill burden may have hindered adherence, and future studies should focus on improving consumption methods to enhance compliance. However, it has been recommended that ingestion of omega-3 supplements on an empty stomach can decrease the absorption rates due to it being fat-soluble (Shahidi & Ambigaipalan, 2018; Summerton, 2015). These findings suggest that the design of an omega-3 supplementation protocol is crucial in participant adherence.

3.3.3 Omega-3 Supplementation and the effects on Endurance Performances

As previously stated in chapter 3.2.4, a total of *n* = 9 studies utilised a VO_2max protocol within their research design (Ávila-Gandía et al., 2020; Da Boit et al., De Salazar et al., Lewis et al., 2015; López-Román et al., 2019; McAnulty et al., 2010; Nieman et al., 2009; Peoples 2008; Poprzecki et al., 2009). However, only a total of *n* = 4 studies compared performance data from pre- to post- supplementation (Ávila-Gandía et al., 2020; López-Román et al., 2019; Peoples 2008; Poprzecki et al., 2009). Even though Da Boit and colleagues (2015), did not repeat their VO_2max protocol after their supplementation period, it is important to note that these authors set a different resistance increase ($W \cdot min^{-1}$) depending on gender (30 W for males & 20 W for females). Even though, these authors did not provide specific rationale for

this gender-based protocol, Bassett (2002), found that elite women have VO2max values ~ 10% lower than their male counterparts with similar training status. This difference has been hypothesised due to females having a limited capacity to deliver oxygen to the working muscles due to physiological factors such as smaller hearts, lungs, and a lower haemoglobin mass compared to males (Santisteban et al., 2022).

From the findings presented by authors utilising VO_2max (Ávila-Gandía et al., 2020; López-Román et al., 2019; Peoples 2008; Poprzecki et al., 2009), no significant differences were found in the participants time taken (s) to exhaustion (Ávila-Gandía et al., 2020; Poprzecki et al., 2009), VO_2max ($ml \cdot kg \cdot min^{-1}$ [Ávila-Gandía et al., 2020; López-Román et al., 2019; Peoples et al., 2008; Poprzecki et al., 2009]), and power output (Ávila-Gandía et al., 2020; López-Román et al., 2019; Peoples et al., 2008; Poprzecki et al., 2009) from omega-3 supplementation ($p > 0.05$). Poprzecki and colleagues (2009) acknowledged finding no significant differences within any VO_2max performance measure, which agrees with previous research (Bortolotti et al., 2007; Brilla & Landerholm, 1990). However, these studies were excluded from the final paper search due to not having full access to the paper. However, López-Román and colleagues (2019) found a significant decrease in maximal power output (Wmax) within their competitive cyclist group pre- to post- omega-3 supplementation (415 ± 15 vs 412 ± 35 Wmax; $p = 0.02$), compared to the non-competitive group which found a significant increase (355 ± 40 vs 365 ± 28 Wmax; $p = 0.05$). This suggests that training status may influence the effects of omega-3 supplementation, with competitive cyclists experiencing different physiological responses compared to non-competitive cyclists**.** However, these authors did acknowledge that future research should include a larger

population sample to confirm their findings and further explore the impact of training status on omega-3 supplementation outcomes.

Ávila-Gandía and colleagues (2020), found a significant decrease in maximal HR ($b \cdot min^{-1}$) in the omega-3 group from pre- to post- supplementation (186 ± 8 vs 183 ± 9 $b \cdot min^{-1}$; p = 0.008) in comparison to the placebo group (181 ± 10 vs 182 ± 9 $b \cdot min^{-1}$; p = 0.02). This is supported by Peoples and colleagues (2008), who also found a significant decrease in maximal HR in the omega-3 group from pre- to post- omega-3 supplementation (186 ± 2 vs 179 ± 2 $b \cdot min^{-1}$; $p < 0.05$), in comparison to the placebo group (185 ± 2 vs 183 ± 3 $b \cdot min^{-1}$; $p > 0.05$). However, López-Román and colleagues (2019) and Poprzecki and colleagues (2009) found no significant differences of max HR ($b \cdot min^{-1}$) in both their omega-3 and placebo groups, respectively ($p > 0.05$). Maintaining an optimal HR is important for cyclists as it can aid muscular flexibility, strength, and endurance performances (Dong, 2016). However, due to the variation of findings across these studies, no conclusions can be drawn upon the influence of an endurance cyclists HR during a VO_2max protocol.

A previously stated in chapter 3.2.4, a total of n = 6 different endurance tests were used which included a Wingate (Hingley et al., 2017; Macartney et al., 2014), maximal cycling power test (Hingley et al., 2017), work capacity trial (Maccartney et al., 2014), SWEET (De Salazar 2020), TT (Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; Nieman 2009), and SSC (Hingley et al., 2017; Macartney et al., 2014; McAnulty et al., 2010; Nieman et al 2009; Peoples 2008; Poprzecki 2009).

The Wingate protocol found no significant differences in peak power output (Hingley et al., 2017), mean power output (Hingley et al., 2017), rpm (Hingley et al., 2017), and peak HR (Macartney et al., 2014), in both omega-3 and placebo groups from pre- to post-

supplementation, $p > 0.05$. Macartney and colleagues (2014) hypothesised this was due the cyclists exercising at their peak cardiovascular effort during each test. Although, the Wingate test is usually performed once in a session (Greer et al., 1998; Hazell et al., 2010), it has been suggested that repeating the sprints four, five, or six times could increase the aerobic power, capacity, and maximal aerobic capacity in athletes Hazell et al., 2010. Therefore, this may be the reason for no significant differences were found. Hingley and colleagues (2017) found no significant differences in power during the maximal cycling power test (W) from pre- to post-omega-3 supplementation in both their omega-3 and placebo groups ($p > 0.05$). Furthermore, Macartney and colleagues (2014) found no significant difference in peak HR ($b.min^{-1}$) during the work capacity trial in both their omega-3 and placebo groups from pre- to post-supplementation ($p > 0.05$). Collectively, these findings suggest that omega-3 supplementation does not affect maximal cycling power or peak HR during work capacity trials**.** However, De Salazar and colleagues (2020) found a significant decrease in oxidative stress from pre- to post- supplementation in the placebo group and the groups receiving 350 mg and 1050 mg of omega-3 group from the SWEET protocol ($p < 0.05$). However, the groups receiving 1750 mg and 2450 mg of omega-3 per day, found no significant differences from pre- to post- supplementation ($p > 0.05$). This suggests that omega-3 dosages between 350 to 1050 mg per day might be effective in reducing oxidative stress. However, as the placebo group also experienced this decrease in oxidative stress, it creates the possibility that well-trained cyclists may benefit from no omega-3 supplementation, indicating a placebo effect. Due to the limited number of studies utilising these protocols, no conclusions can be made as to whether omega-3 supplementation can benefit a Wingate, maximal cycling power test, work capacity trial(s), and SWEET protocols, respectively.

The data presented by the authors Da Boit and colleagues (2015), Hingley and colleagues (2017), Lewis and colleagues (2015), and Nieman and colleagues (2009), found no significant differences in the Wmax (Hingley et al., 2017), HR (Da Boit et al., 2015), and time to completion (Da Boit et al., 2015; Lewis et al., 2015; Nieman 2009), during the TT from pre- to post- supplementation ($p > 0.05$). Da Boit and colleagues (2015) were the only researchers to measure HR ($b \cdot min^{-1}$) during a TT and therefore no conclusions of the effect of omega-3 on this variable can be made. However, Nieman and colleagues (2009), found a significant difference ($p < 0.05$) in relative oxygen consumption from pre- to post-omega-3 supplementation (no pre-supplementation data vs 64.2 3.2 $ml \cdot kg \cdot min^{-1}$). This suggests that omega-3 may improve cycling economy, which is a significant component to an endurance cyclists' performance (Vikmoen et al., 2016). However, only one study found this result and therefore further research is needed to confirm this potential benefit. Furthermore, Nieman and colleagues (2009), conducted the 10-km TT within their final 15- min of their SSC, however, these authors did not provide rationale for this protocol design. Palmer and colleagues (1997), investigated the effects of 150-- min of ether steady state (58% of peak power output) or stochastic cycling (58 ± 12.2% of peak power output), during a 20 km TT in six competitive cyclists. Finding that TT times were significantly faster following an SSC (26:32 ± 1:30 min) in comparison to stochastic cycling (28: 08 ± 1:47 min), $p < 0.05$. These suggest that greater TT performances may be achieved if the TT were performed immediately after an SSC, therefore, future studies should explore this hypothesis within an endurance cycling population.

The findings by Hingley and colleagues (2017), Macartney and colleagues (2014), McAnulty and colleagues (2010), Nieman and colleagues (2009), Peoples (2008), and

Poprzecki (2009), found no significant differences in resting oxygen consumption (Hingley et al., 2017; McAnulty et al., 2010; Poprzecki 2009), HR (Macartney et al., 2014; McAnulty et al., 2010; Nieman et al 2009; Poprzecki 2009), and power (McAnulty et al., 2010; Nieman et al 2009; Peoples 2008; Poprzecki 2009) from pre- to post- supplementation in both omega-3 and placebo groups during a SSC ($p > 0.05$). However, Peoples, and colleagues (2008) found a significant decrease in peak HR within their omega-3 group from pre- to post-supplementation (186 ± 2 vs 179 ± 2 $b \cdot min^{-1}$; $p < 0.05$) in comparison to the placebo group which found no significant difference (185 ± 2 vs 183 ± 3 $b \cdot min^{-1}$; $p > 0.05$). As only one study found a positive HR outcome during an SSC protocol, further research is needed to support this finding. Hingley and colleagues (2017) found that absolute VO_2 was not significantly different from pre- to post-omega-3 supplementation, 1.65 ± 0.05 vs 1.68 ± 0.02 $L \cdot min^{-1}$ ($p > 0.05$). Furthermore, finding no significant differences between the omega-3 and placebo groups results ($p > 0.05$). Similarly, McAnulty and colleagues (2010) found the mean VO_2 to be similar across multiple supplementation groups (placebo 2589 ± 180, vitamin and mineral 3025 ± 153, omega-3 2725 ± 170, and omega-3 with vitamin and mineral 2846 ± 193 $ml \cdot min^{-1}$, $p = 0.6$). However, this study did not include a pre- supplementation SSC therefore, no conclusions can be made whether omega-3 could benefit VO_2 results during SSC protocols. The differences in distances and power outputs across these studies may be due to the varying training status of the participants. Therefore, future research is needed to determine if SSC protocols are only beneficial for specific training experiences.

3.3.4 Omega-3 Supplementation and the effects on Neuromuscular (NMF)

As previously mentioned in chapter 3.2.5, a total of *n* = 9 tests were used to cause NMF, this included; MVC (Hingley et al 2017; Lewis et al., 2015), electrical stimulations (Lewis et al., 2015), maximal back squat jumps (Lewis et al., 2015), CMJ (Lewis et al., 2015), push ups (Lewis et al., 2015), with the following protocols used to confirm the presence of fatigue, using; urine (De Salazar et al 2020), saliva (Nieman et al., 2009), and blood (Ávila-Gandía et al., 2020; Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; Macartney et al., 2014; McAnulty et al., 2010; Nieman et al., 2009; Peoples et al., 2008; Poprzecki 2009), samples.

Hingley and colleagues (2017), found a significant decrease in maximal force post exercise during the MVC protocol, from pre- to post- supplementation in both the omega-3 (234 ± 18 vs 225 ± 12 Nm, $p < 0.05$) and placebo groups (236 ± 12 vs 215 ± 15 Nm, $p < 0.05$). This suggests that omega-3 supplementation has no protective or enhancing effect upon a cyclist's muscle during a MVC protocol. This is supported by Lewis and colleagues (2015), who found no significant differences in MVC force from pre- to post- supplementation in both the omega-3 and placebo groups ($p > 0.05$). However, Lewis and colleagues (2015), found a very likely beneficial increase in muscle activation from electrical stimulations from pre- to post-supplementation in the omega-3 group (22.0 % ± 20.0; OR = 763; $p < 0.05$) in comparison to the placebo group (−11.3 % ± 12; OR = 0; $p > 0.05$). This is ideal for endurance cyclists who are aiming for stronger muscular strength (Maffiuletti et al., 2000). However, the practicality of using electrical stimulations regularly can be questioned, as the risk of injury and overtraining associated with this technique may outweigh the potential benefits, making this less suitable for endurance training (Alon, 2013; Teschler & Mooren, 2019). However, Lewis and colleagues (2015) found no significant differences from pre- to post- supplementation in CMJ height and

maximal number of push ups completed in both the omega-3 and placebo groups ($p > 0.05$.), respectively. As Lewis and colleagues (2015) were the only authors to utilise measures of NMF with a cycling population, further research is needed to validate these protocols. Furthermore, a broader body of evidence is needed to establish which protocol is optimal to identify and measure NMF in endurance cyclists.

De Salazar and colleagues (2020) found a significant decrease in oxidative stress 24 h post exercise in the participants urine samples from pre- to post-supplementation (1391 ± 1768 vs 1157 ± 1428 ng·kg at 24 h) in the placebo and 350 mg omega-3 per day groups ($p < 0.05$). However no significant differences ($p > 0.05$) were found in the 1050 mg, 1750 mg, and 2450 mg omega-3 per day groups. This suggests that low dosages of omega-3 (> 250 mg per day for 4 weeks) may be beneficial in reducing oxidative stress in endurance cyclists. Furthermore, Nieman and colleagues (2009) found no significant differences of Ig A (protein in the participants saliva samples) from pre- to post-supplementation, immediately post-exercise, and 14 h post- exercise in both the placebo and omega-3 groups ($p > 0.05$). As Ig A is used to gauge the immune systems response to exercise (Gleeson, 2007), these results suggest that omega-3 does not affect an endurance cyclists' immune function. However, these studies method of analysis (urine and saliva), were not consecutively utilised by the other researchers found in this search (Ávila-Gandía et al., 2020; Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; López-Román et al., 2019; Macartney et al., 2014; McAnulty et al., 2010; Peoples et al., 2008; Poprzecki 2009), therefore, blood sample analysis remains the more common method of assessing physiological changes in endurance cyclists.

Most studies (Ávila-Gandía et al., 2020; Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; Macartney et al., 2014; McAnulty et al., 2010; Nieman et al., 2009; Peoples et al.,

2008; Poprzecki 2009) utilised a blood analysis protocol from the antecubital/median cubital vein. However, Ávila-Gandía and colleagues (2020), was the only study to collect blood samples using the capillary method. This is significant as de Oliveira and colleagues (2018), investigated the reliability of plasma total CK activity in capillary and venous blood samplings in soccer players (*n* = 22) after a training session, finding that capillary blood samplings cannot be a reliable alternative to venous sampling as it could lead to incorrect data interpretation by sports practitioners. A total of n = 4 studies (Hingley et al., 2017; McAnulty et al., 2010; Nieman et al., 2009; Poprzecki 2009), did not specify the volume of blood taken during the samples. It is important for researchers to justify blood collection volumes, so it does not interfere with the athlete's performance outcomes, such as power output and maximal exercise duration (Christensen & Christensen, 1978; Haller et al., 2023). Furthermore, only two studies provided specific time points for pre- exercise blood samples (McAnulty et al., 2010; Nieman et al., 2009), despite studies providing these timepoints post- exercise (Ávila-Gandía et al., 2020; Da Boit et al., 2015; McAnulty et al., 2010; Nieman et al., 2009; Poprzecki 2009). To date there is no specific guidance on how long an athlete should wait to exercise after bloods sampling. However, Panebianco and colleagues (1995), investigated the effects of blood donations (1 unit [87.8 ml]) on exercise performance (VO_2max and ventilatory threshold) in male amateur cyclists *(n* = 10). Finding that VO_2max significantly affected for at least 1-week after blood donation (4854 ± 209; 2 h post 4454 ± 228; 2-days post 4464 ± 187; 7-days post 4506 ± 217 $ml \cdot kg \cdot min^{-1}$, $p < 0.05$). This highlights the negative effects large volume blood samples can have on cycling performance. However, it is worth acknowledging that the studies found (Ávila-Gandía et al., 2020; Da Boit et al., 2015; Lewis et al., 2015; Macartney et al., 2014; Peoples et al., 2008) in this search did not take large quantities of bloods from their participants, which should therefore negate some impact on performance.

A total of n = 14, different blood markers were used to analyse the effects of omega-3 supplementation. No significant differences were found in erythrocyte fatty acid compositions from pre- to post- supplementation in both the placebo and omega-3 groups ($p > 0.05$, Da Boit et al., 2015; Hingley et al., 2017; Lewis et al., 2015; Macartney et al., 2014; Peoples et al., 2008). However, a significant increase was found in erythrocyte DHA within the omega-3 group from pre- to post-omega-3 supplementation in (1) Hingley and colleagues (2017) no data provided, $p < 0.05$, (2) Macartney and colleagues (2014) 4.05 ± 0.2 vs 5.63 ± 0.2 AU, $p < 0.05$, and (3), Peoples and colleagues (2008), 8.36 ± 1.03 vs 11.82 ± 2.4, $p < 0.05$. Macartney and colleagues (2014) stated this then resulted in a significant increase ($p < 0.05$) in the omega-3 index within the omega-3 group (4.7 ± 0.2 vs 6.3 ± 0.3 AU) in comparison to the placebo group (4.2 ± 0.2 vs 3.9 ± 0.2 AU, $p > 0.05$). Furthermore, Lewis and colleagues (2015) found a significant increase ($p = 0.004$) in erythrocyte EPA from pre- to post-supplementation in the omega-3 group (0.41 ± 0.16 vs 0.68 ± 0.27 AU) in comparison to the placebo group (0.59 ± 0.34 vs 0.49 ± 0.24, $p > 0.05$). Research by McAnulty and colleagues (2010) found no significant differences in plasma EPA and DHA as a result from the supplementations of omega-3, placebo, vitamin, vitamin and omega-3 ($p > 0.05$). However, this was not supported by Nieman and colleagues (2009), as they found a significant increase ($p < 0.05$) in plasma EPA and DHA within their omega-3 group (311% and 40% above placebo [$p > 0.05$]) from pre- to post- supplementation. However, this finding failed to have any significant effect upon their performance outcomes. McAnulty and colleagues (2010) found a significant decline in ORAC from baseline to post- exercise in the placebo, omega-3, vitamin, omega-3 and vitamin supplementation groups ($p \leq 0.001$). However, the pattern of change was not significantly different between the groups ($p = 0.2$), suggesting that these supplementation protocols had no impact upon ORAC during endurance cycling. The same

authors also found a significant increase ($p \leq 0.017$) from baseline to post-exercise in ferric-reducing antioxidant potential within the vitamin group (no data provided). However, no significant differences were found in the placebo, omega-3, and vitamin and omega-3 groups ($p > 0.05$). These findings suggest that omega-3 supplementation (2000 mg per day for 6-weeks) has no effect on antioxidant intake. However, supplements with vitamin C (2000 mg), vitamin E (800 IU), vitamin A (3000 IU) and selenium (200 µg) per day for 6-weeks can increase a cyclist's antioxidant capacity, potentially reducing muscle damage and fatigue during cycling performances (Clemente-Suárez et al., 2023). Therefore, it should be explored whether a lower or higher dosage and/or duration of omega-3 could further increase these benefits.

Ávila-Gandía and colleagues (2020) found no significant differences in lactate from pre- to post- supplementation in both the omega-3 (880.6 ± 874.5 vs 908.1 ± 157.2 AU, $p = 0.8$) and the placebo (908.1 ± 157.2 vs 918.4 ±127.0 AU, $p = 0.7$) groups post-exercise. This suggests that 1220 mg of omega-3 for 30-day does not effectively enhance muscular fuel sources in endurance cyclists. McAnulty and colleagues (2010) found a significant increase in F_2-isoprostanes from baseline to post- exercise in the omega-3 group (53 %; $p < 0.05$), in comparison to the placebo, vitamin, and vitamin and omega-3 groups, respectively ($p > 0.05$). Furthermore, Da Boit and colleagues (2015) found a significant increase in natural killer cell cytotoxic activity (no data provided) and peripheral blood mononuclear cells (no data provided) from pre- to post- supplementation during the recovery period (3 h post-exercise), in the omega-3 group ($p < 0.05$) in comparison to the placebo group ($p > 0.05$). However, these same authors (Da Boit et al., 2015) found no significant differences in thiobarbituric acid reactive substances from pre- to post- supplementation in both the placebo and omega-3 groups ($p > 0.05$). These findings suggest that omega-3 supplementation may increase

oxidative stress, potentially leading to muscle damage negatively impacting endurance cycling performances (Burt & Twist, 2011) However, Poprzecki and colleagues (2009) found no significant effects upon muscle cell damage from pre- to post-exercise plasma CK activity in both the omega-3 and placebo groups ($p > 0.05$), suggesting that 1300 mg of omega-3 for 6-weeks cannot effectively indicate muscle damage caused by endurance cycling, therefore further research is needed to explore variations in omega-3 dosages and durations to determine more effective strategies in indicating muscle damage using blood markers.

Poprzecki and colleagues (2019) found no significant differences in resting and post-exercise uric acid levels from pre- to post- supplementation in both the omega-3 and placebo groups ($p > 0.05$). Furthermore, Nieman and colleagues (2009) found no significant differences from pre- to post- supplementation for both the omega-3 and placebo groups in plasma IL-1ra ($p = 0.7$), plasma IL-6 ($p = 0.7$), and plasma IL-8 ($p = 0.7$). These findings are supported by Da Boit and colleagues (2015), who also failed to find any significant differences in plasma IL-6 from pre- to post-supplementation in both the placebo and omega-3 groups ($p > 0.05$). Collectively, these findings suggest that omega-3 supplementation does not significantly reduce markers inflammation following endurance exercise in cyclists. However, as the placebo groups also found no significant differences, it could be inferred that the exercise protocols used by these authors (Da Boit et al., 2015; Nieman et al., 2009; Poprzecki et al., 2019) were not effective enough to generate inflammation in a cycling population, therefore, it is possible neither of these supplementations could produce measurable results. However, due to the limited studies exploring inflammation and omega 3, more studies are needed to expand the knowledge-base.

3.3.5 Secondary Testing

A total of n = 4, studies included nutritional analysis during their supplementation period (Ávila-Gandía et al., 2020; Da Boit et al., 2015; De Salazar et al., 2020; Poprzecki et al., 2009). However, Da Boit and colleagues (2015) failed to present their findings on participant dietary intake, therefore no analyses can be drawn from this study. Of the three studies that did report dietary intakes, no significant differences were found in macronutrient (CHO, protein, fat, and omega-3) and energy intakes between the omega-3 and placebo groups across the supplementation periods ($p > 0.05$, Ávila-Gandía et al., 2020; De Salazar et al., 2020; Poprzecki et al., 2009). Indicating that the participants across both supplementation groups shared similar dietary habits.

Furthermore, no significant differences were found in the intake of omega-3 between both groups (omega-3 vs placebo, $p > 0.05$, Ávila-Gandía et al., 2020; Peoples et al., 2008; Poprzecki et al., 2009). This is an important finding as it suggests that those who were in the 53.1% of the placebo group (47.9 %) consumed enough omega-3 in their habitual diet to match the omega-3 intakes in the group who consumed the additional supplement(s), or there was an absorption issue within the omega-3 groups. Consequently, this lack of dietary restrictions may have led to the placebo group consuming similar levels of omega-3, potentially masking the true effects of the supplementation or the possibility that omega-3 offers no effect within a cycling population. Therefore, future researchers should implement stricter dietary controls, such as restricting omega-3 intake, to ensure more accurate assessments of omega-3's effect on an endurance cycling populace can be made. Additionally, considering the nutritional analysis (which accounts for the participants time burden) and stricter dietary tracking to monitor participant adherence during the supplementation phase.

3.3.6 Limitations and Bias

To minimise the selection bias associated with the non-randomisation of participants, only randomised trials were included in this systematic review. This analysis was performed using the PEDro scale. Out of the initial pool of studies identified through the search strategy, 11 papers met the inclusion criteria and therefore included in the review. However, the small number of papers found could be seen as a limitation, as the small number of studies can reduce the reliability of the data, as there is less evidence to support the findings (Guyatt et al., 2008). However, this does highlight a gap in endurance cycling and omega-3 supplementation research.

As part of the PEDro checklist, authors had to specify whether participants were randomised into groups, if allocation was concealed, and whether both subjects and assessors were blinded to this allocation. However, Poprzecki and colleagues (2009) did not specify if participant allocation was concealed and stated that the participants and administrators were not blinded to the process. The lack of blinding may create a possible selection bias (Mansournia et al., 2017), therefore the allocation of participants could have influenced the outcomes found by the authors. Also, López-Román and colleagues (2019) used single-centred open labelled design for their supplementation protocol. Therefore, due to the lack of blinding, it could suggest a bias towards the internal validity, such as participants over or under reporting outcomes as a result from the supplementation consumption/ingestion (Wartolowska et al., 2017).

The studies included in the systematic review lacked the inclusion of females in their populations (*n* = 20) compared to males (*n* = 269). However, McAnulty and colleagues (2010), did not specify gender distribution amongst their participants (*n* = 48). The lack of gender-specific data prevents conclusions about any gender differences regarding omega-3 supplementation and cycling. Understanding these differences are important to distinguish as it allows for an advanced individualisation of the supplement's benefits and enhancing scientific reproducibility (Schilaty et al., 2018). Furthermore, all participants were over the age of 18 with the mean age of 25.8 (5.3) years. This is significant as when individuals age, they might experience physiological changes which could influence their athletic performance (Tanaka & Seals, 2003). Therefore, dosage, duration, and possible co-ingestion of omega-3 supplementation may need to be adjusted to suit the requirements of an older populace (Murphy & McGlory, 2021). Consequently, no conclusions could be drawn of the effect of omega-3 on specific age ranges. As previously stated, the participants training experiences were varied across these studies.

3.3.7 Conclusion and Future Research

This systematic search found that ~1.05 $g \cdot day^{-1}$ was the lowest dose to start producing positive results within endurance exercises, with a neutralisation trend at the highest does of 2.45 $g \cdot day^{-1}$ in amateur cyclists (De Salazar et., 2020). Furthermore, training status was found to affect an endurance cycling performance from pre- to post-omega-3 supplementation. With López-Román and colleagues (2019), identifying a significant decrease in maximal power output within their competitive cyclist group pre- to post-omega-3 supplementation (415 ±

15 vs 412 ± 35 Wmax; p = 0.02), compared to the non-competitive group which found a significant increase (355 ± 40 vs 365 ± 28 Wmax; p = 0.05).

Also, HR appeared to provide varying results across the different endurance protocols, and therefore no conclusions can be made whether or not omega-3 influences HR in endurance cyclists' performance. Furthermore, as there was a limited number of studies utilising endurance (Wingate, maximal cycling power test, work capacity trials, and SWEET) protocols and NMF (MVC, electrical stimulations, maximal back squat jumps, CMJ, and push ups) tests, no additional conclusions can be made regarding the effectiveness of omega-3. However, omega-3 supplementation (>250 $mg \cdot day^{-1}$ for 4-weeks) was found to be beneficial in reducing oxidative stress in endurance cyclists (De Salazar et al., 2020).

Lewis and colleagues (2015) recommended that participants are to be recruited from a similar athletic background to ensure that the results are relevant to specific training types or training, such as amateur cyclists. Furthermore, Nieman and colleagues (2009), suggested enhancing the research design by extending the supplementation period (>6-weeks). Longer supplementation durations would improve the quality of data, providing more robust evidence regarding the effects of omega-3 supplementation in cyclists. Peoples and colleagues (2008), suggested that time to fatigue (used in their protocol) as a performance indicator produced too much subject variability. Therefore, it was recommended to use cycling TT, due to its increased reliability to quantify endurance performances (Peoples et al., 2008), thus providing a greater insight into how omega-3 affects endurance cycling performances.

A further research consideration should address the high pill burden faced by participants during Phillips and colleagues (2021) study. Furthermore, it is important to avoid

consuming omega-3 supplements on an empty stomach (Shahidi & Ambigaipalan, 2018; Summerton, 2015). Therefore, this study will seek to address some of these limitations by providing participants with a 250 ml omega-3 drink to be consumed alongside their breakfasts. Moreover, in studies where participants dietary intake was monitored during the supplementation period (Ávila-Gandía et al., 2020; Da Boit et al., 2015; De Salazar et al., 2020; Poprzecki et al., 2009), found no significant differences in omega-3 intakes were found between the omega-3 and placebo groups ($p > 0.05$). This study will aim to implement strict monitoring of dietary intakes, including specific limits on omega-3 and macronutrients (protein, CHO, and fat) relative to their body weight. This approach aims to ensure an accurate assessment of omega-3 supplementation effects and to prevent confounding factors related to dietary variability.

Chapter 4. Intervention Design

4.1 Method

4.1.1 Ethical Approval

This research protocol received approval from the Faculty of Health Sciences local ethics committee (REF 22-23.50) at the University of Hull prior to the commencement of any testing.

4.1.2 Participants

A total of nine non-professional road cyclists and triathletes were initially recruited for this study (age 40.3 [15.5] years; height 179.2 [7.9] cm; body mass 75.8 [7.7] kg). However, due to participant withdrawals, only six participated in this study (age 45.7 [34.8] years; height 182.5 [176.7] cm; body mass 80 [4.5] kg). The participants were characterised as "trained" (McKay et al., 2021). Recruitment strategies included a recruitment poster (see Appendix E), which was advertised in the local area, and word of mouth referrals from participants who shared information about the study within their cycling community.

Participants who were recruited local cycling clubs and word-of-mouth were required to meet the following inclusion criteria: (1) currently following a training plan at the start of the study, (2) had a minimum of two years' experience of training and racing, (3) had not suffered any illness or injury in the previous 6-months (including COVID-19 infection) and (4) were not currently taking or had taken any dietary supplements in the preceding 6-months. Exclusion criteria included (1) voluntary withdrawal from the study, (2) being vegan, and (3)

having a known allergy or intolerance to omega-3 or a known adverse event to omega-3 supplementation. A full explanation of the trial protocol, including the studies purpose, was provided in writing via the Participant Information Sheet (PIS) located in Appendix F. All participants subsequently signed the written informed consent (see Appendix F), agreeing with the study protocol and their voluntary participation.

Participants were informed of their right to withdraw from the study at any time without providing a reason. All data collected up to that point was kept unless specifically requested otherwise, which then it was destroyed. All personal information and data were kept confidential throughout the research process. Participants were allocated an anonymous participant number, and their personal details were stored separately to ensure that data remained anonymous. All information collected in this study was stored in accordance with the Data Protection Act (2018).

4.1.3 Experimental Design

Participants were asked to travel to the Exercise Physiology Research laboratory at the University of Hull on six separate occasions between June and September (2023). The protocol of each visit was as follows: (1) Visit one and six: participants underwent anthropometrical profiling (including ISAK 8 site skinfold assessment) and a VO_2max assessment, (2) visit two and four: consisted of a 75-minute SSC at 60% Wmax, followed by a 16.1 km TT. During these visits, NMF was indirectly assessed through a CMJ protocol at three points (pre- SSC, post-SSC, and post-TT), and (3) visit three and five: venous blood samples 24 h post the completion of the exercise protocols on visits two and four.

Visit three and four were interspersed by a 56 day/8-week omega-3 and protein beverage supplementation period (1600 mg omega-3; 820 mg DHA and 550 mg EPA; + 20 g protein [Enhanced Recovery Omega-3^{TM} drink, Felicity Nutrition Ltd, Bagshot, UK]), taken daily. Upon completion of the supplementation period, participants returned for visits four, five, and six. The studies protocol design can be found in Figure 3 (below).

Participants were instructed to refrain from any strenuous activity 48 h before each visit. Additionally, they were encouraged to attend sessions in a euhydrated state (20 ml of fluid per kg of body weight), fed (2 g of carbohydrates per kg of body weight). Participants were also asked to abstain from any sources of caffeine 24 h before any visit. To assist their dietary requirements, participants were provided with standardised breakfast suggestions tailored for a 70 kg cyclist. Examples included: (1) rolled oats (80 g), full fat milk (200 ml), honey (2 teaspoons), banana (118 g), raisins (20 g), and orange juice (500 ml), 2) granola (140 g) full fat milk (100 ml), honey (2 teaspoons), banana (118 g), and kiwi (69 g), and 3) shredded wheat (3 biscuits), full fat milk (150 ml), orange juice (500 ml), banana (118 g), honey (2 teaspoons), dates (32 g). Participants were also provided with information on how to adapt these suggestions according to their specific body weight to ensure they met dietary requirements.

Upon each visit, the researcher briefed the participants on the sessions procedures and addressed any questions or concerns they had. Once satisfied, participants were presented with both the pre-exercise medical questionnaire (including some brief supplementation history questions) and the informed consent form (see Appendix G & H). These documents were signed before any testing commenced.

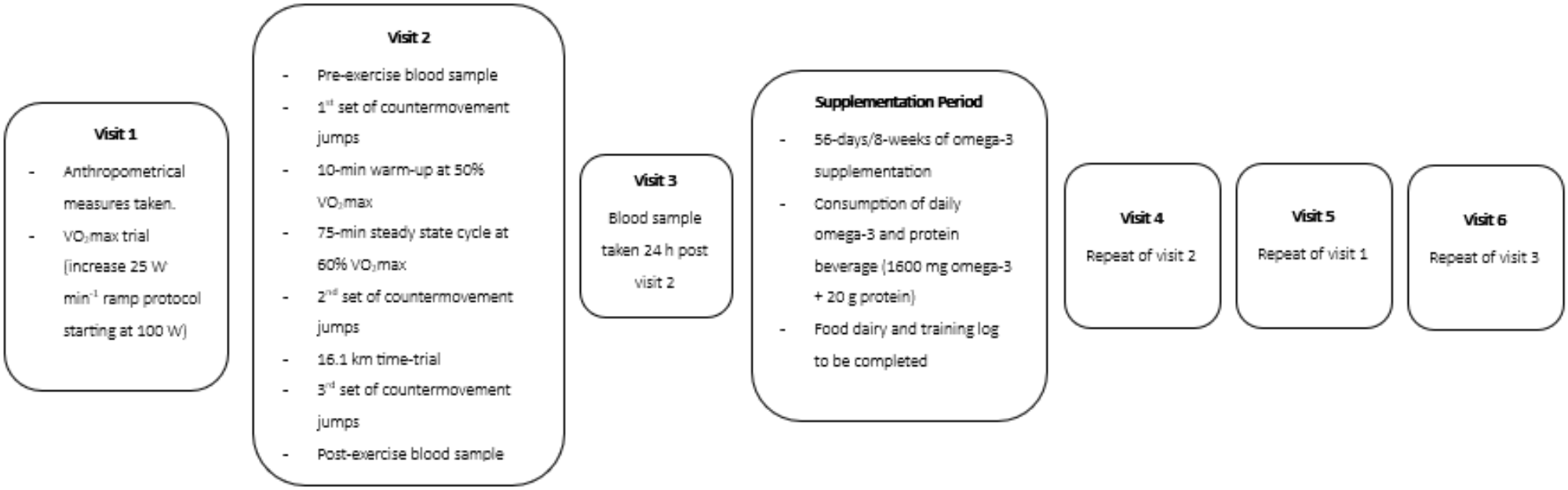

Figure 3. Study design protocol and outline

4.1.4 Anthropometrical Profiling

Prior to each VO_2max test, participants had their anthropometrical measures taken, including weight and height. Height was measured using a wall-mounted stadiometer (Seca, Vogel and Halke, Hamburg, Germany). Participants stood barefoot with heels together and backs and shoulders in contact with the stadiometer. Height was recorded to the nearest 0.1 cm after the participant had taken a deep breath. Weight was measured to the nearest 0.1 kg (Seca balance, Vogel and Halke, Hamburg, Germany), with participants not wearing shoes.

After height and weight measurements, participants underwent a calliper skinfold evaluation of subcutaneous adipose tissue in accordance with the Internation Society for the Advancement of Kinathropomerty (ISAK; Stewart, Marfel-Jones, Olds & De Ridder, 2011). Initial marking of sites was done using a Lufkin Executive Thinline 2 m anthropometric tape, (Baltimore, USA). Skinfold measurements were taken at eight sites: triceps, subscapular, bicep, supraspinal, iliac crest, abdomen, mid-thigh, and medical calf, using Harpenden Skinfold Callipers (London, UK). A researcher grasped two parallel layers of skin and subcutaneous tissue at each marked site using their index finger and thumb. The callipers were applied 1 cm away from the marked site, perpendicular to the skin surface, and measurements were taken 2 s after calliper application. A minimum of two measurements were taken, and if they were within ± 7.5%, the average was recorded. All skinfold measurements were conducted by an ISAK-accredited level 1 anthropometrist.

4.1.5 Maximal Oxygen Uptake (VO_2max) Protocol

The VO_2max test was conducted on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode B.V., Groningen, Netherlands), which was adjusted to match the participant's bicycle dimensions and fitted with their personal pedal system. Breath-by-breath cardiopulmonary data was collected using a Cortex Metalyzer 3B (Cortex Biophysik, Leipzig, Germany) during a graded exercise test. Metabolic variables (VO_2, VCO_2, and V_E) were measured and recorded using the Cortex, interfaced with a PC installed with Metasoft Studio software (Cranlea Human Performance, Birmingham, England). Prior to each test, the Cortex was calibrated for volume measurements using a 3-L air syringe (model 5530, Hans Rudolf, Kansas City, USA). A 2-point gas calibration was also performed by sampling ambient air and calibration gas with known quantities of O_2 and CO_2. Immediately prior to the graded exercise test, a 1-point gas calibration was completed.

Participants completed the VO_2max test using a graded exercise protocol, starting at 100 W and increasing 25 W every min until volitional exhaustion or a plateau in VO_2 despite increased exercise intensity, with a respiratory exchange ratio > 1.05 (Devereux et al., 2022; Hebisz et al., 2018). Participants were asked to maintain a cadence between 70 to 120 revolutions per minute (rpm). Every 2-min, participants reported their rating of perceived exertion (RPE) through the Borg scale (Borg, 1982).

During the VO_2max test, ventilatory data were continuously captured and averaged over 15-sec time periods using Metasoft Studio software. The software also collected HR (b·min-1) data from a Bluetooth Polar HR monitor (H10. Polar Electro, Kempele, Finland). All data were exported and stored in an Excel spreadsheet (Office 365, Microsoft, Washington, USA). All tests were conducted under controlled environmental conditions, temperature

22.2°C (1.3°C), humidity of 49.7 % (9 %), and barometric pressure 1006 HPa (7.7 HPa). A standard fan set at high speed was used to cool the participants throughout testing.

These measures were repeated after the 56-day/8-week supplementary period (visit 6).

4.1.6 Steady State Cycle (SSC), Time trial (TT), and Countermovement Jump (CMJ) Protocols

Upon arrival, participants were provided with the same size mask and HR monitor (size obtained from visit 1) which was connected to the Cortex Metalyzer, which underwent the same calibration procedures (see Chapter 4.1.5). Once comfortable, the participants positioned themselves on the cycle ergometer and completed a 10-min warm-up at 50% of their Wmax, which was identified from visit one.

Once the warm-up was completed, participants removed their shoes to perform the first CMJ test battery. All collections of CMJ data were made using the same iPhone 12 (iOS 16 Apple Inc., Cupertino, CA, USA) high speed camera by the same evaluator, who had no prior experience with video analysis. The app used to calculate the CMJs was developed using XCode 5.0.5 for Mac OSX 10.9.2 (App) and designed to leverage the iPhone 12's 120 Hz high-speed camera at 720 p resolution. The app, *My Jump Lab* (V 4.4.1) calculated the flight time of the CMJ by identifying the take-off and landing frames of the recording. Jump height was then determined using the equation $h = t^2 \times 1.22625$ (Bosco et al., 1983), where h represents height (m), and t represents flight time (s).

The evaluator maintained a consistent position, standing 1.5 m away from the participant, as per the app's calibration instructions, with the iPhone 12 facing the participant. The CMJ protocol included participants performing three vertical jumps by starting in a static standing position with hands on their hips and legs straight during the flight phase of the jump (Haekkined & Komi, 1985). They then squatted to a 90-degree angle and jumped as high as possible, ensuring that the landing was performed with ether their feet maintaining ankle dorsiflexion. The peak value of the jumps was recorded, and each jump was separated by a 60 s rest period.

Next, participants were repositioned back onto the cycle ergometer and reconnected to the Cortex system to perform the 75-min SSC at 60% of their Wmax. During this part of the trial, participants were allowed to listen to music as it would not affect their performance data. Throughout the test, HR, peak oxygen O_2, RER, and metabolic variables were collected automatically by the Cortex system. In five-minute intervals, participants were asked to rate their RPE using the Borg scale (Borg, 1982).

After the 75-min submaximal exercise period, participants were instructed to stop listening to any music and their mask removed. During the subsequent 5-min rest period, participants were instructed to complete the second set of the CMJ protocol. Following this, participants were instructed to complete the 16.1 km TT as quickly as possible, with only the distance variable visible to them on the Wattbike (Wattbike Ltd, Nottingham, United Kingdom). Participants continued to wear the chest HR monitor, with HR recorded at 2 km intervals during the TT. Additionally, participants reported their RPE after every 2 km using Borg scale (Borg, 1982). Time to completion, speed, and power output at the end of the TT

was collected and downloaded using the specific Wattbike Hub software (V 6.3.0). After the TT, participants then completed the final CMJ protocol.

Visits two and four was separated by a 56-day/8-week supplementation phase.

4.1.7 Supplementation Phase

The supplementation phase lasted for 56-days/8-weeks. During this period participants were instructed to consume the omega-3 and protein beverage (Enhanced Recovery Omega-3™). Each pre-mixed, single serving 250 ml beverage contained 1600 mg of omega-3 (820 mg DHA and 550 mg EPA) and 20 g protein. This beverage contained apple, pear, and blackcurrant juices from concentrate, whey protein (milk), fish oil (cod), collagen hydrolysate, > 2% pectin, black cumin oil, l-carnitine, sunflower seed protein, natural and artificial flavourings, rosemary extract, mixed tocopherols, and vitamin D. Importantly, this product is batch tested and complies with Informed Sport certification, providing athletes and researchers with a level of quality assurance (Informed Sport Certified Product Search, 2023).

To ensure adherence to the supplementation protocol, participants were given written reminders along the supplements. These reminders included: (1) instructions to consume the supplement daily with breakfast, (2) not to consume any additional omega-3 supplements that were not provided by the researchers, and (3) not to consume > three servings of fish per week (~500 mg of omega 3 daily). Additionally, participants received weekly contact from a researcher, with the reminder to consume the supplements to reinforce compliance. These measures were implemented to maintain consistency in the supplementation process throughout the 56-days/8-week period.

4.1.8 Diet and Activity Control

Participants were instructed to maintain a food diary (weighed is possible) at fixed periods during the trial (see Appendix I). The details the participants were required to record included: (1) time of consumption (e.g. breakfast, lunch) and (2) to describe the food types that were consumed. To allow direct comparisons and ensure consistency, participants were required to record their food intake over the same 72 h period each week (including twice on a weekday and once on a weekend). Participants were provided with weekly reminders (via email or SMS) to keep the food diary up to date.

To ensure participants did not proceed the omega-3 limit set for this study, they were provided with a list of foods which were naturally high omega-3 foods (> 500 mg per serving). Some examples included oily fish, green leafy vegetables, kidney beans, chia seeds, and walnuts (Calder & Yaqoob, 2009; Miedzianka et al., 2017; Simopoulos, 2002). A list of fortified omega-3 foods and drinks was also provided. This included bread, fruit juices, eggs, milk, oils, yogurt, and butter/spreads (Feizollahi et al., 2018; Ganesan et al., 2014; Shahidi & Ambigaipalan, 2016). Participants were advised that omega-3 content in these foods could vary by brand and shop, and they were encouraged to check food labels carefully to ensure they did not exceed the omega-3 limit. All food diaries were analysed for its calorific and macronutrient content using Nutritics dietary analysis software and using the present UK/Irland database (Nutritics student edition V 6.0; Swords, Ireland).

Similarly, participants were provided with a training log (see Appendix J), for them to provide details of their training loads during the 56-day/8-week of omega-3 supplementation period. Participants were instructed to record the duration (min), average and maximum HR

($b \cdot min^{-1}$), activity type, and the weekly summated Training Stress Score ([TSS], if available). They were instructed to complete this every time they took part in training, with weekly reminders sent (via email or SMS), to aid compliance.

4.1.9 Illness and Wellness Questionnaire

Before the participants performed their SSC, 16.1 km TT, and CMJ protocol during visits 2 and 4, they were asked to complete the athlete wellbeing questionnaire and an illness questionnaire (see Appendix K and L). The athlete wellbeing questionnaire asked the participants to score their perceived fatigue, sleep quality, general muscle soreness, stress levels, and mood on a scale of 1 to 5, with 5 being the most positive. The illness questionnaire asked participants if they had any injuries or faced any difficulties since starting the research study.

The questionnaires were included as part of the overall study protocol to monitor the participants wellbeing and health, ensuring that any changes could be accurately assessed. To allow direct comparisons, they were completed at the same day and time each week.

4.1.10 Data Analysis

All statistical analysis were completed using JASP software (JASP Team 2023, v0.17.1) and presented as mean (SD), unless otherwise stated. Repeated measures analysis of variance (ANOVAs) was used to compare ergo-spirometry parameters collected during the VO_2max test, SSC, and TT both pre- and post- the 56-day (8-week) supplementation period.

Paired sample t-tests were conducted to analyse CMJ heights (cm) at three different timepoints (pre-SSC, post-SSC, and post-TT) both pre- and post-omega-3 supplementation. Additionally, *Post hoc* analyses were performed to determine effect size differences in CMJ heights (cm) under the same measures, using Cohen's *d* (2013). T-tests were also used to analyse athletes' skinfolds, completed food diaries, training logs, and athlete wellbeing questionnaires. These was performed to quantify participant body weight, total macronutrient intake (protein, CHO, fat, and omega-3), training duration and maximum HR, and athlete wellbeing scores. Prior to the analysis, data were screened to ensure all assumptions were met.

Mean differences between variables were used to assess the changed in outcome variables. Confidence intervals (CI) were utilised to measure uncertainty within the sample variables, with results at the 95% CIs considered the true mean value. Significance was accepted at $p < 0.05$.

Although it is generally accepted that an *a priori* estimation of the minimal sample size for adequate statistical power be reported (Harriss, MacSween, & Atkinson, 2019), the time constraints and modest participant burden (e.g., training and food logs, daily ingestion of omega-3 beverage) in this thesis made such an estimation unfeasible (Lakens, 2022). As a result, only a *Post hoc* statistical power estimation has been provided, which is typically discouraged and have limited value (Harriss, MacSween, & Atkinson, 2019; Sullivan, & Feinn, 2012).

Based on the guidelines set out by Lakens (2022), who suggests that a power of 90% is preferable for a single study, the analysis was conducted using JASP (v0.17.1) with an

observed effect size (Cohen's *d* = 0.5), an alpha level of 0.05, and a two-tailed paired samples t-test. A sample size of 44 was found to provide a 90% power to detect a medium effect size.

Chapter 5. Results

5.1 Skinfolds

There were no significant changes in the mean sum of the 8 skinfolds (mm) from pre- to post-omega-3 supplementation. The skinfolds were pre: 82.8 (28.6) vs post: 86.8 (33.8) mm; $F(1, 5) = 1.7$; $p = 0.3$.

5.1.1 Individual Skinfold Sites

Further investigation also showed there were no significant differences found at any of the individual skinfold sites pre- to post- omega-3 supplementation (see Table 4). Triceps pre: 9.2 (3.4) vs post: 9.4 (3.7) mm; $F(0.3, 5) = 0.6$; $p = 0.7$, subscapular pre: 11.5 (4.7) vs 11.8 (4.0) mm; $F(0.2, 5) = 0.5$; $p = 0.5$, biceps pre: 4.5 (1.4) vs post: 5.2 (1.7) mm; $F(0.1, 5.0) = 0.7$; $p = 0.7$, lilac crest pre: 14.7 (6.5) vs post: 15.5 (9.9) mm; $F(1.7, 3) = 0.3$; $p = 0.2$, supraspinal pre: 9.9 (4.5) vs post: 11.0 (4.7) mm; $F(1.2, 5) = 0.3$; $p = 0.3$, abdominal pre: 16.4 (5.3) vs post: 17.0 (8.6) mm; $F(1.6, 5) = 0.3$; $p = 0.3$, front thigh pre: 9.9 (4.1) vs post: 10.2 (4.1) mm; $F(0.7, 5) = 0.5$; $p = 0.2$, and medical calf pre: 6.5 (2.7) vs post: 7.4 (3.7) mm; $F(0, 5) = 0.9$; $p = 0.9$.

Table 4. The mean difference and 95% CI in the sum of eight sites from pre- to post-omega-3 supplementation

	Triceps (mm)	**Subscapular (mm)**	**Bicep (mm)**	**Lliac Crest (mm)**	**Supraspinal (mm)**	**Abdominal (mm)**	**Front Thigh (mm)**	**Medical Calf (mm)**
Mean difference	-0.2	0.3	-0.1	-1.7	-0.5	-1.3	-1.6	-0.02
95% CI	-0.9 to 0.7	-0.8 to 1.3	-0.4 to 0.3	-4.9 to 1.6	-1.7 to 0.7	-4 to 1.4	-4.1 to 0.9	-0.7 to 0.6
Significance (*p*)	0.7	0.5	0.7	0.2	0.3	0.3	0.2	0.9

*Statistically significant difference ($p < 0.05$)

5.2 Maximal Oxygen Uptake (VO_2max)

There were no significant differences in the time taken (s) to reach volitional exhaustion during the VO_2max test from pre- to post- omega-3 supplementation. Time to reach volitional exhaustion were pre: 682.9 (177.1) vs post: 716.1 (122.6) s; $F(1, 5) = 1.7$; $p = 0.2$ (see Table 5).

There were also no significant differences found in VO_2max values ($ml \cdot kg \cdot min^{-1}$) reached during the study duration. Maximal oxygen uptake values were pre: 54.0 (4.7) vs post: 52.0 (6.6) $ml \cdot kg \cdot min^{-1}$; $F(1, 5) = 2.3$; $p = 0.2$ (see Figure 4 and Table 5).

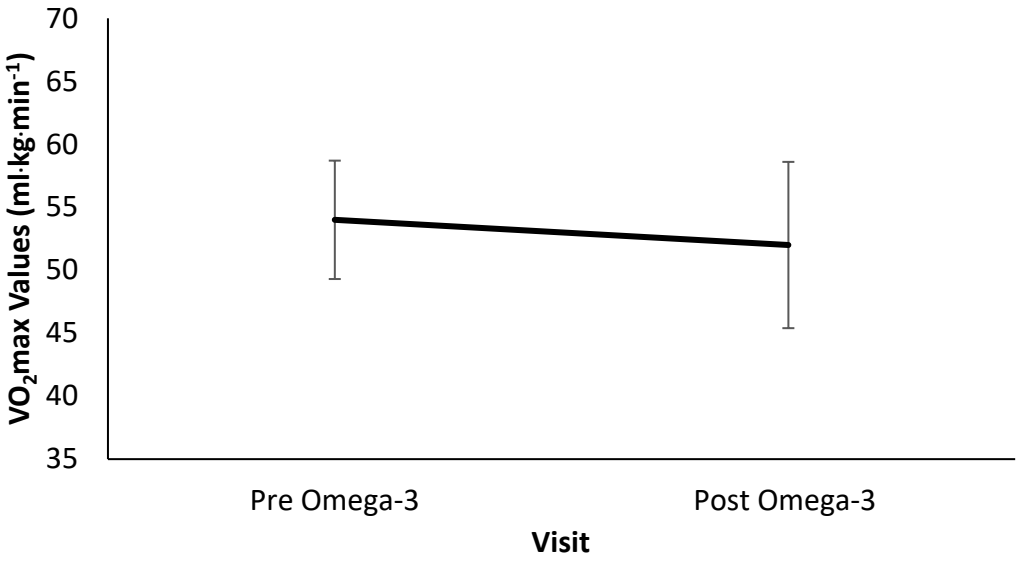

Figure 4. Changes in mean time to complete (s) during a 16.1 km TT from pre- to post-omega-3 supplementation with each participant, including the SD.

There were no significant differences found in the power achieved at VO_2max (Wmax) from pre- to post- omega-3 supplementation. The power achieved at VO_2max was pre: 392.3 (65.3) vs post: 399.8 (54.6) Wmax; $F(1, 5) = 1.8$, $p = 0.3$ (see Table 5).

Finally, there were no significant differences in the maximal HR achieved in the VO_2max test (HRmax) from pre- to post- omega-3 supplementation. The HR achieved at VO_2 max was pre: 173 (20) vs post: 173 (14) $b \cdot min^{-1}$; $F(1, 5) = 0.0$; $p = 1.0$ (see Table 5).

Table 5. The mean difference and 95% CI in the time taken, VO_2max, power at VO_2max, and HR at VO_2max achieved by participants at volitional exhaustion from pre- versus post-omega-3 supplementation

	Time (s)	**VO_2max ($ml \cdot kg \cdot min^{-1}$)**	**Power at VO_2max (Wmax)**	**HRmax ($b \cdot min^{-1}$)**
Mean difference	-33.1	1.7	-7.5	0
95% CI	-98.2 to 31.9	-1.1 to 4.5	-21.9 to 6.9	-7.1 to 7.1
Significance (*p*)	0.2	0.2	0.3	1.0

*Statistically significant difference ($p < 0.05$)

5.3 Steady State Cyle (SSC)

There were no significant differences in the mean VO_2 ($ml \cdot kg \cdot min^{-1}$) achieved during the SSC, found pre- to post- omega-3 supplementation. The mean VO_2 achieved were pre: 41 (4.1) vs post: 36.9 (6.1) $ml \cdot kg \cdot min^{-1}$; $F(1, 5) = 3.7$; $p = 0.1$ (see Table 6).

No significant differences were also found in the mean HR ($b \cdot min^{-1}$) achieved during the SSC from pre- to post- omega-3 supplementation. The HR achieved during the SSC was pre: 153 (18) vs post: 139 (8) $b \cdot min^{-1}$; $F(1, 5) = 2.9$, $p = 0.1$ (see Table 6).

Table 6. The mean difference and 95% CI in both the mean VO_2 and mean HR achieved by the participants during the SSC pre- to post-omega-3 supplementation

	Mean VO_2 ($ml \cdot kg \cdot min^{-1}$)	**Mean HR ($b \cdot min^{-1}$)**
Mean difference	1.9	13.5
95% CI	-0.2 to 1.7	-6.8 to 33.7
Significance (*p*)	0.1	0.1

*Statistically significant difference ($p < 0.05$)

5.4 Time Trial (TT)

There were no significant differences in the time taken (s) to complete the 16.1 km TT from pre- to post- omega-3 supplementation. Time taken to complete the 16.1 km TT was pre: 1510.9 (144.3) vs post: 1397.0 (101.5) s; $F(1, 5) = 4.1$; $p = 0.09$ (see Figure 5 and Table 7).

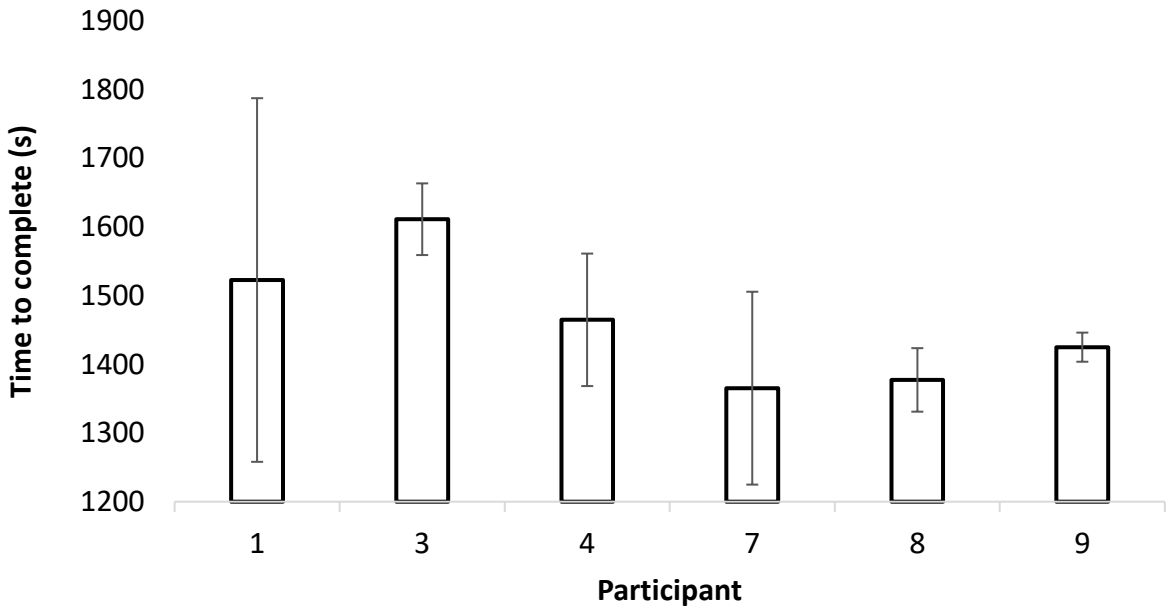

Figure 5. Changes in mean time to complete (s) during a 16.1 km TT from pre- to post-omega-3 supplementation with each participant, including the SD.

No significant differences were also found in the peak power (Wmax) achieved during the 16.1 km TT from pre- to post- omega-3 supplementation. The power achieved was pre: 367.0 (113.2) vs post: 422.2 (198.7) W; $F(1, 5) = 0.3$; $p = 0.6$ (see Figure 6 and Table 7).

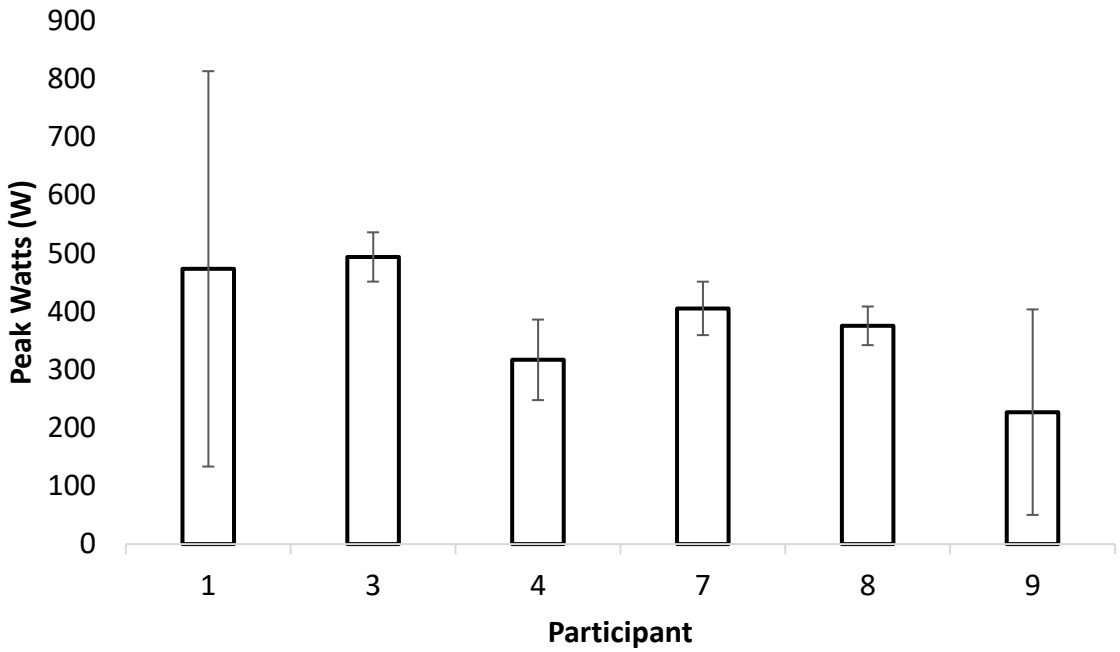

Figure 6. Changes in mean peak power (Wmax) during a 16.1 km TT from pre- to post-omega-3 supplementation with each participant, including the SD.

Finally, there were no significant differences found in maximal HR ($b \cdot min^{-1}$) achieved during the 16.1 km TT (HRmax) from pre- to post- omega-3 supplementation. The HRmax achieved during the 16.1 TT were pre: 165 (12) vs post: 161 (16) $b.min^{-1}$; $F(1, 5) = 2.3$; $p = 0.2$ (see Figure 7 and Table 7).

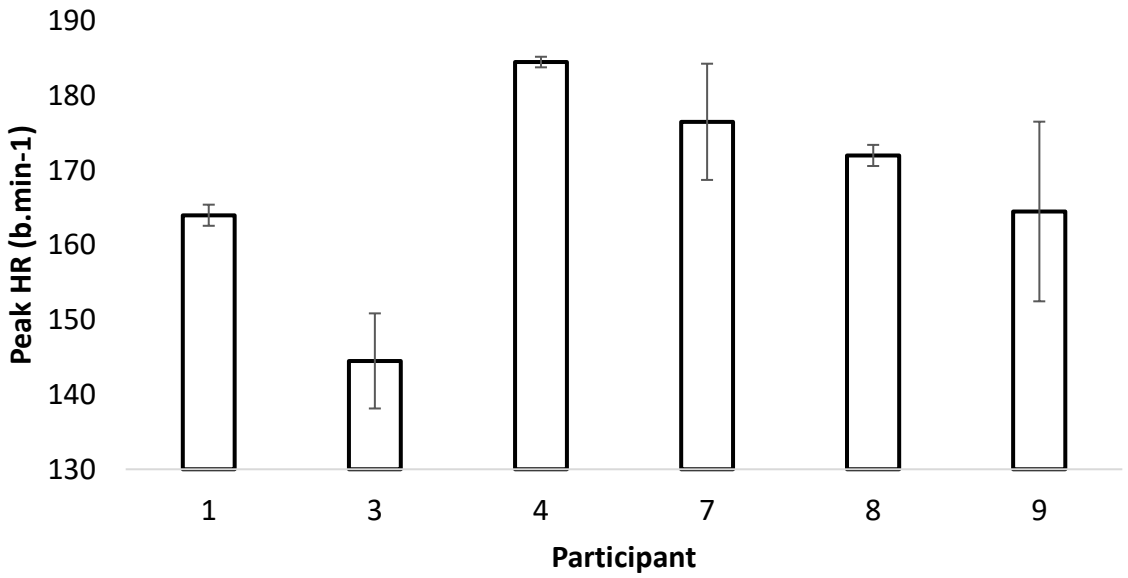

Figure 7. Changes in mean HRmax ($b \cdot min^{-1}$) during a 16.1 km TT from pre- to post-omega-3 supplementation with each participant, including the SD.

Table 7. The mean difference and 95% CI in the time taken, power, and HR achieved during the 16.1 km TT from pre- versus post- omega-3 supplementation

	Time to complete (s)	**Peak Power (W)**	**Peak HR ($b \cdot min^{-1}$)**
Mean difference	113.9	-55.2	4.5
95% CI	-30.9 to 258.8	-308.4 to 198.1	-3.1 to 12.1
Significance (*p*)	0.09	0.6	0.2

*Statistically significant difference ($p < 0.05$)

5.5 Countermovement Jump (CMJ)

There were no significant differences between the peak heights (cm) achieved during the CMJ protocol both pre- and post- omega-3 supplementation. The heights achieved were pre: 37.5 (8.4) vs post: 39.0 (3.7) cm; $F(1, 5) = 0.3$; $p = 0.6$ (see Table 8). The changes in CMJ height both pre- and post-omega-3 supplementation are presented in Figures 8 and 9, respectively. Furthermore, there was no significant differences found between the CMJ timepoints (pre to post SSC, pre to post TT, and post SSC to post TT) throughout the exercise protocol from pre- and post- omega-3 supplementation (see Table 8).

Table 8. The mean difference and 95% CI and effect size differences of the change in CMJ height (cm) at Pre, Post SSC, and Post TT both pre- to post-omega-3 supplementation

	Pre	Post SSC	Post TT	Significance (*p*)	Effect Size (η_p^2)
Pre- omega-3 supplementation (cm)	39. 5 (7.4)	36.4 (8.9)	36.6 (9.6)	$F(2, 5) = 3.0$, $p = 0.1$	0.4
Post- omega-3 supplementation (cm)	40.9 (4.3)	38.5 (3.0)	37.5 (6.4)	$F(1, 5) = 3.0$, $p = 0.1$	0.4

Post hoc analysis	Pre to Post SSC				Pre to Post TT				Post SSC to Post TT			
	Mean difference (± 95% CI)	*t*	Sig (*p*)	Effect size (*d*)	Mean difference (± 95% CI)	*t*	Sig (*p*)	Effect size (*d*)	Mean difference (± 95% CI)	*t*	Sig (*p*)	Effect size (*d*)
Pre- omega-3 supplementation (cm)	3.1 (-1.5 to 7.6)	1.7	0.1	0.7	2.9 (-1.6 to 7.3)	1.7	0.2	0.7	-0.2 (-3.9 to 3.5)	-0.1	0.9	0.2
Post- omega-3 supplementation (cm)	2.5 (-1.2 to 6.1)	1.7	0.1	0.7	3.4 (-4.2 to 11.0)	1.2	0.3	0.5	0.9 (-3.7 to 5.6)	0.5	0.6	0.2

*Statistically significant difference ($p < 0.05$). Observed effect magnitudes are: trivial (< 0.3), small (0.31 to 0.5), moderate (^{M}), large (^{L}), very large (^{VL})

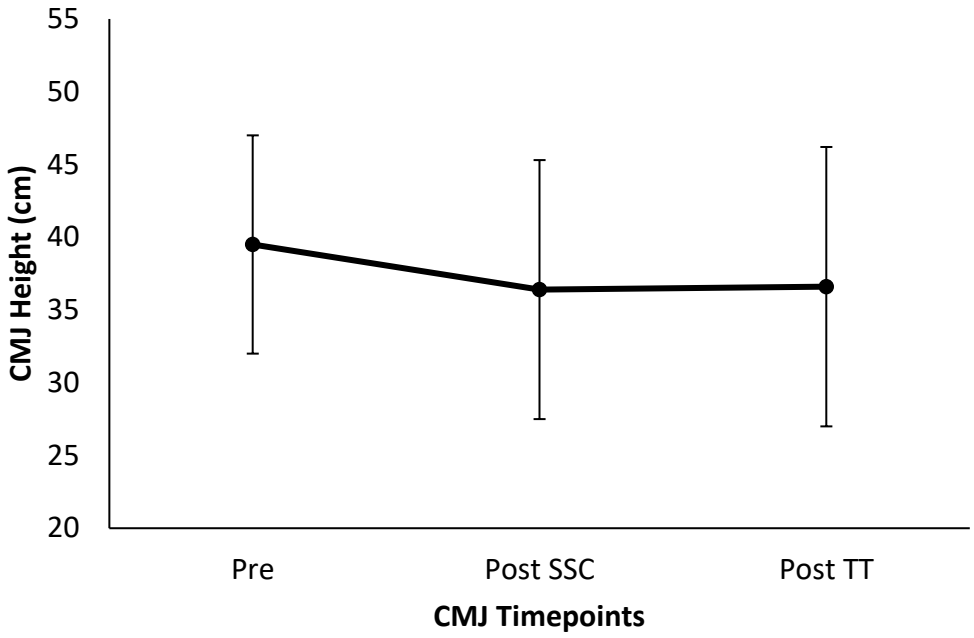

Figure 8. Changes in mean CMJ height (cm) pre- omega-3 supplementation at each time point (pre, post SSC, and post TT), including the SD.

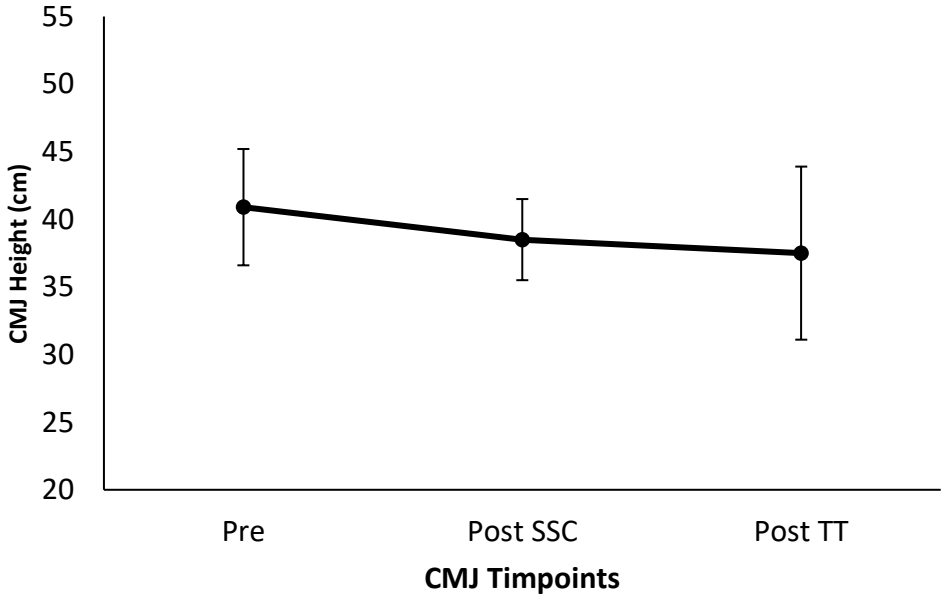

Figure 9. Changes in mean CMJ height (cm) post- omega-3 supplementation at each time point (pre, post SSC, and post TT), including the SD.

Table 9. The mean difference and 95% CI of the CMJ heights (cm) produced throughout the exercise protocol (Pre to Post SSC, Pre to Post TT, and Post SSC to Post TT) pre- to post- omega-3 supplementation

	Pre to Post SSC	**Pre to Post TT**	**Post SSC to Post TT**
Mean difference	0.5	-0.6	-1.1
95% CI	-5.6 to 6.8	-11.4 to 10.2	-8.4 to 6.1
Significance (*p*)	0.8	0.9	0.7

*Statistically significant difference ($p < 0.05$)

5.6 Food Diary

Over the eight weeks, the mean macronutrient intakes were protein 102.3 (16.3) g, CHO 266.3 (92.7) g, and fat was 83.4 (27.0) g. The mean omega-3 intake was 1.7 (0.1) $g \cdot day^{-1}$ (see Table 10).

Table 10. The mean participant protein (g), CHO (g), fat (g), and omega-3 (g) per day (over the 56-day/8-week supplementation period)

	Participant 1	Participant 3	Participant 4	Participant 6	Participant 8	Participant 9
Protein (g)	100.3 (20.7)	90.9 (28.1)	124.5 (32.2)	120.6 (34.8)	87.9 (18.6)	89.7 (113.3)
CHO (g)	352.5 (58.6)	183.7 (83.9)	357.1 (71.0)	334.8 (162.3)	220.5 (65.1)	149.3 (49.0)
Fat (g)	128.3 (26.4)	59.8 (40.7)	70.7 (51.2)	77.3 (34.2)	103.1 (39.5)	61.3 (23.5)
Omega-3 (g)	1.7 (0.2)	1.9 (0.5)	1.6 (4.5)	1.6 (0.0)	1.7 (0.2)	1.6 (0.02)

5.6.1 Macronutrient and Omega-3 Analysis

There were no significant differences found between any of the participants weekly macronutrient intakes over the 56-day/8-week supplementation period. The mean CHO intake was 266.3 (92.7) g, $F(7, 35) = 1.2$; $p = 0.3$. Also, there were no significant differences found in the CHO intake from week 1 to week 8, during the supplementation period (week 1: 2685.7 [105.9] vs week 8: 266.7 [122.2] g, $F[1, 5)] = 0.8$, $p = 0.4$ [see Figure 10]). Finally, there were no significant differences were found between the participants weekly fat (g) intakes over the supplementation period. The mean fat intake was 83.4 (27.0) g, $F(7, 35) = 0.4$; $p = 0.9$. Furthermore, no significant differences found from week 1 to week 8 (week 1: 78.2 [32.6] vs week 8: 86.9 [34.7] g, $F[1, 5] = 0.1$, $p = 0.1$ [see Figure 11]). The

mean protein intake was 102.3 (16.3) g, $F(7, 35) = 1.2$; $p = 0.3$, with no significant differences found from week 1 to week 8 (week 1: 14.0 [16.8] vs week 8: 23.9 [28.8] g, $F[1, 5] = 0.01$, p =0.9 0.9 [see found from week 1 to week 8 (week 1: 14.0 [16.8] vs week 8: 23.9 [28.8] g, $F[1, 5] = 0.01$, $p = 0.9$ [see Figure 12]).

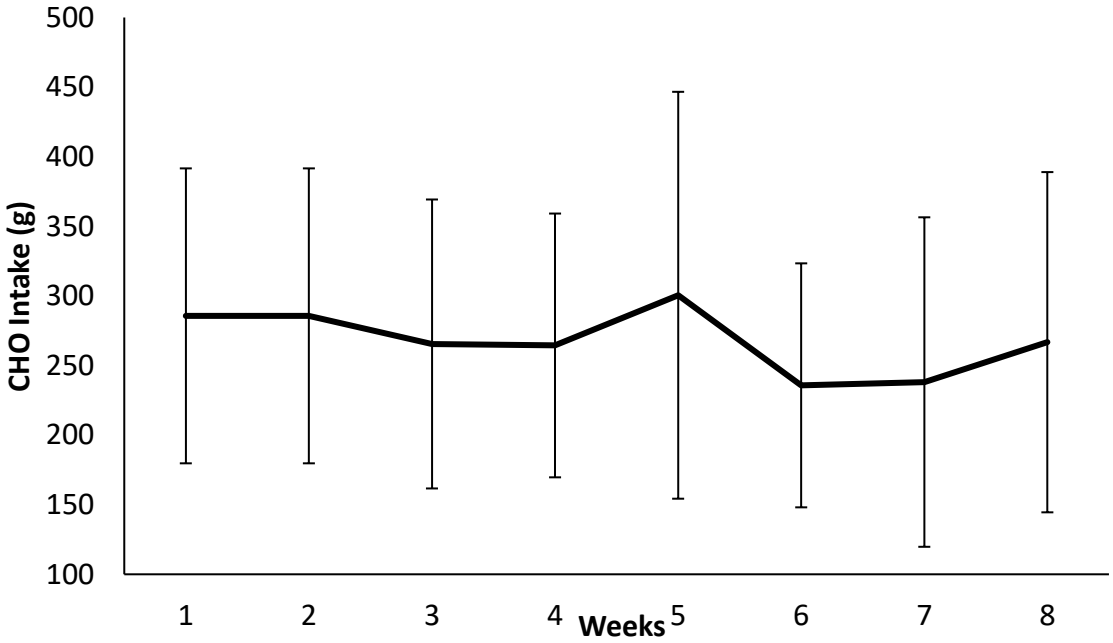

Figure 10: The mean weekly CHO intake over the 8-week omega-3 supplementation period, including the SD.

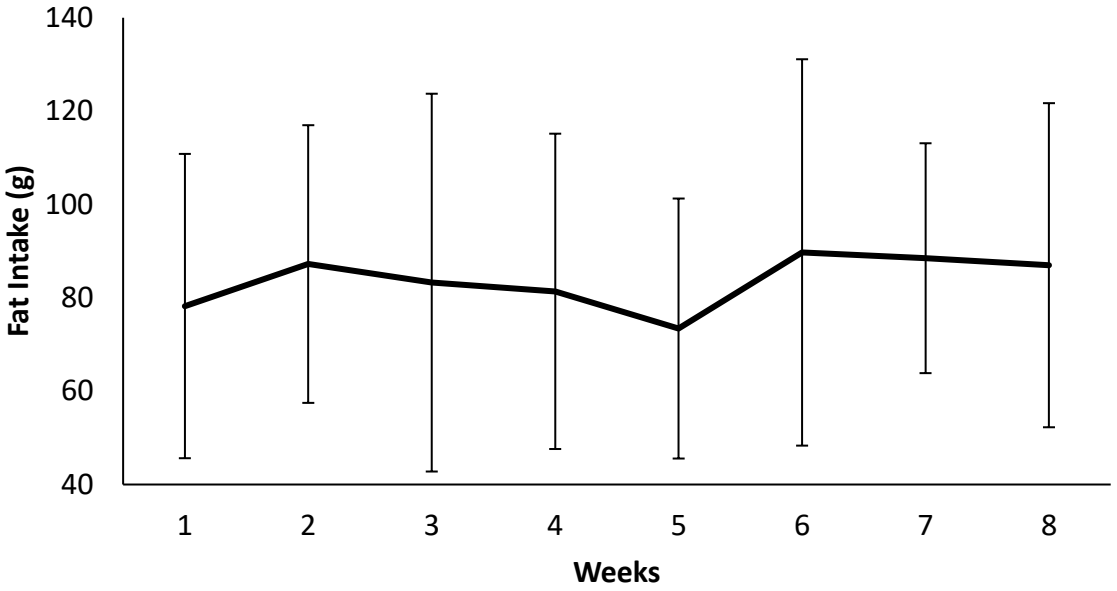

Figure 11: The mean weekly fat intake over the 8-week omega-3 supplementation period, including the SD.

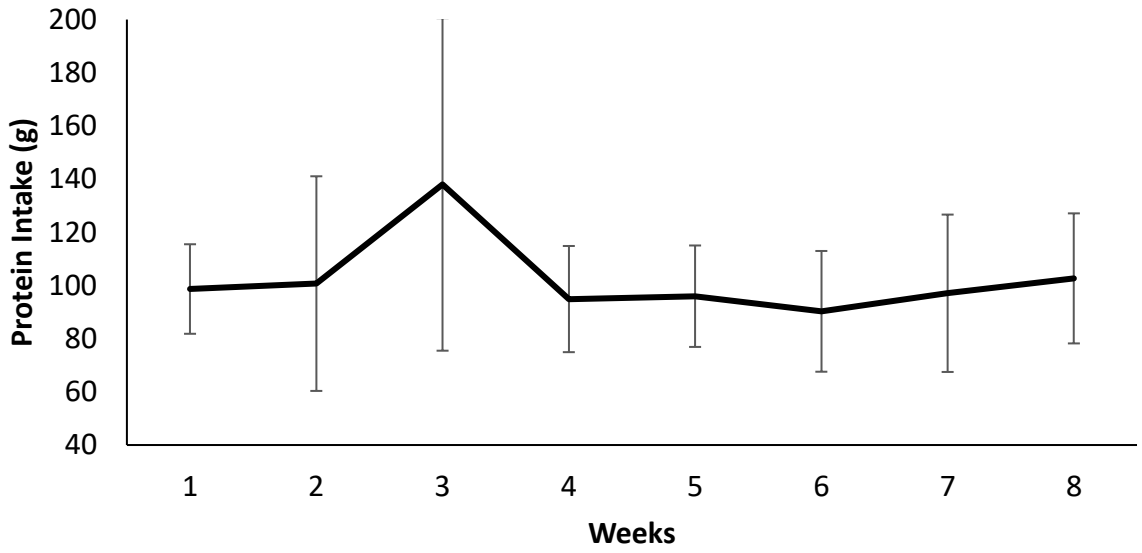

Figure 12: The mean weekly protein intake over the 8-week omega-3 supplementation period, including the SD.

5.7 Training Log

The mean days of activity were 4.0 (2.3) days for cycling, 0.6 (1.1) days for running, 0.1 (0.2) days for swimming, 0.1 (0.4) days for circuit training, and 0.3 (0.8) days per week for walking, individual participant data can be found in Table 11. Furthermore, no significant difference were found in number of days logged for training over the 56-day/8-week omega-3 supplementation period ($F[5, 20] = 0.1$; $p = 0.9$).

Table 11. Participants mean days of activity (cycling, running, swimming, circuit training, and walking) per week, over the supplementation period

	Participant 1	Participant 3	Participant 4	Participant 6	Participant 8	Participant 9
Cycling	5.5 (2.4)	1.3 (0.7)	6.6 (1.2)	5.75 (0.5)	3.5 (0.9)	1.6 (0.9)
Running	0	2.6 (1.4)	0	0	0	1.3 (0.9)
Swimming	0	0.4 (0.5)	0	0	0	0
Circuit Training	0	0	0	0	0	0.9 (0.4)
Walking	0	0	0	0	1.85 (2.6)	0

5.7.1 Duration and Heart Rate (HR) Analysis

There were no significant differences found in the participants mean duration (min) of activity per week over the omega-3 supplementation period. The mean duration of activity

was 496.1 (217.1) min, $F(7, 35) = 0.5$; $p = 0.9$ (see Figure 13). Furthermore, no significant differences were found from week 1 to week 8 (week 1: 542.5 [217.2] vs week 8: 558.2 [187.9] min, $F[1, 5] = 0.1$, $p = 0.8$).

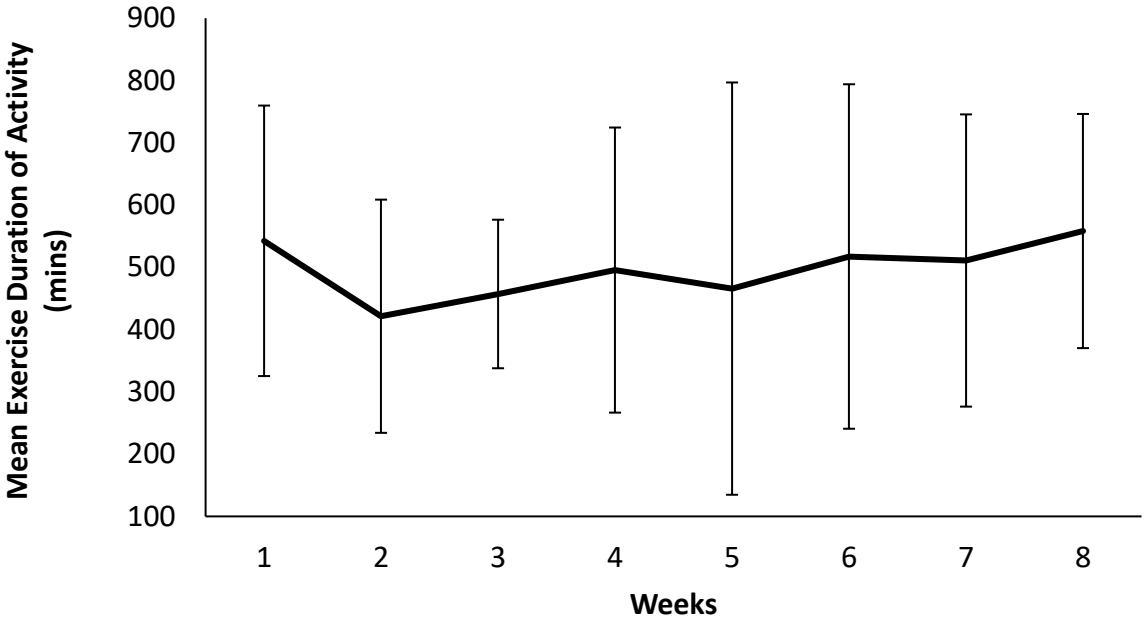

Figure 13: Mean weekly exercise duration of activity (min) over the 56-day/8-week omega-3 supplementation period, including the SD.

Furthermore, there were no significant differences found between the participants mean HR ($b \cdot min^{-1}$) during logged activities across the 8-week omega-3 supplementation period. The mean HR of activity was 136 (9) $b \cdot min^{-1}$, $F(7, 35) = 27.2$; $p = 0.1$ (see Figure 14), with no significant differences were found from week 1 to week 8 (week 1: 136 [10] vs week 8: 136 [8] $b \cdot min^{-1}$, $F[1, 5] = 0.003$, $p = 0.9$).

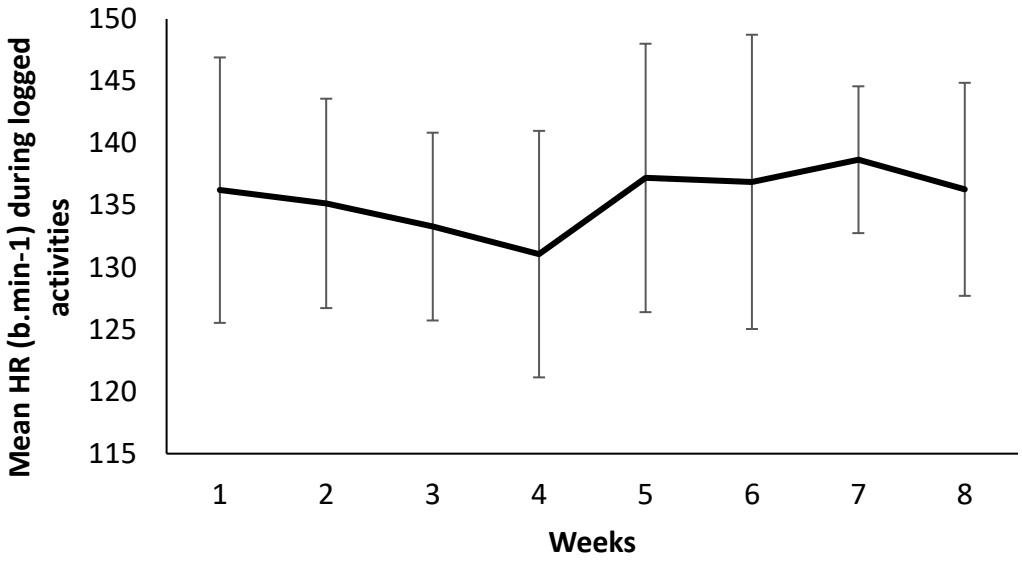

Figure 14: Mean weekly HR ($b \cdot min^{-1}$) during logged activities over the 56-day/8-week omega-3 supplementation period, including the SD.

5.8 Athlete Wellbeing Question

A significant decrease was found in the participants' mean perceived fatigue scores across the omega-3 supplementation period. The mean fatigue score was 3.3 (0.9) AU, $F(7, 35) = 4.8$, $p < 0.001$. However, there were no significant differences from week 1 to week 8 (week 1: 3.8 [1.3] vs week 8: 2.7 [0.8] AU; $F[1, 5] = 2.8$, $p = 0.2$ [see Figure 15]).

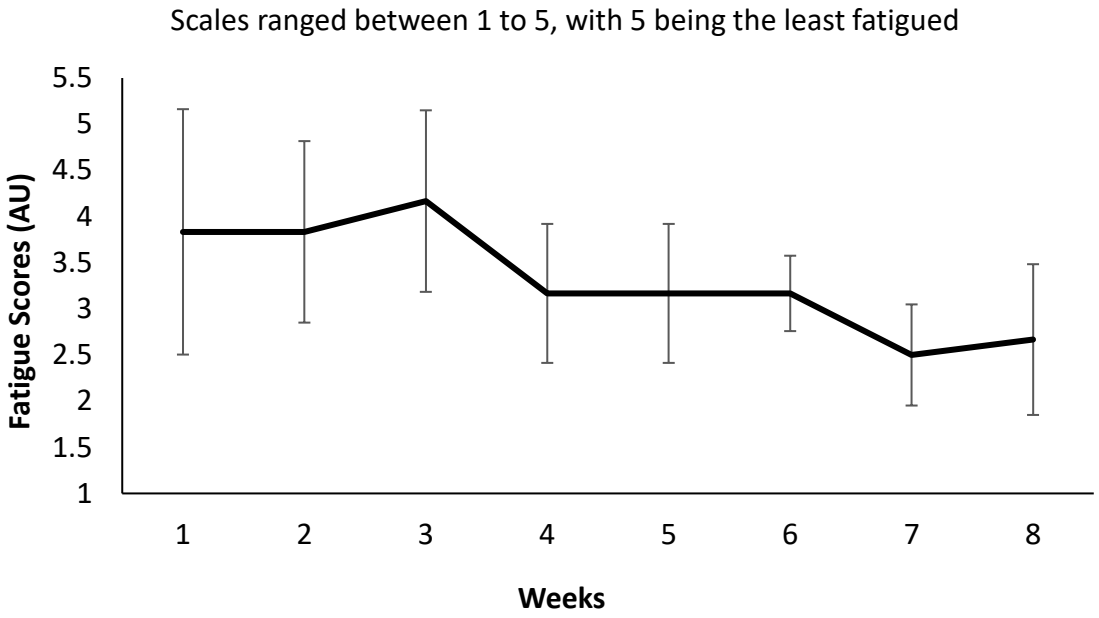

Figure 15: Weekly mean fatigue scores (AU) across the 8-week omega-3 supplementation phase, including the SD.

The participants mean perceived sleep quality scores found a significant decrease across the 8 weeks of omega-3 supplementation. The mean sleep quality score was 3.5 (0.9) AU, $F(7, 35) = 3.1$, $p = 0.01$. However, there was no significant differences from week 1 to week 8 (week 1: 3.7 [0.8] vs week 8: 2.8 [0.9] AU; $F[1, 5] = 4.3$, $p = 0.09$ [see Figure 16]).

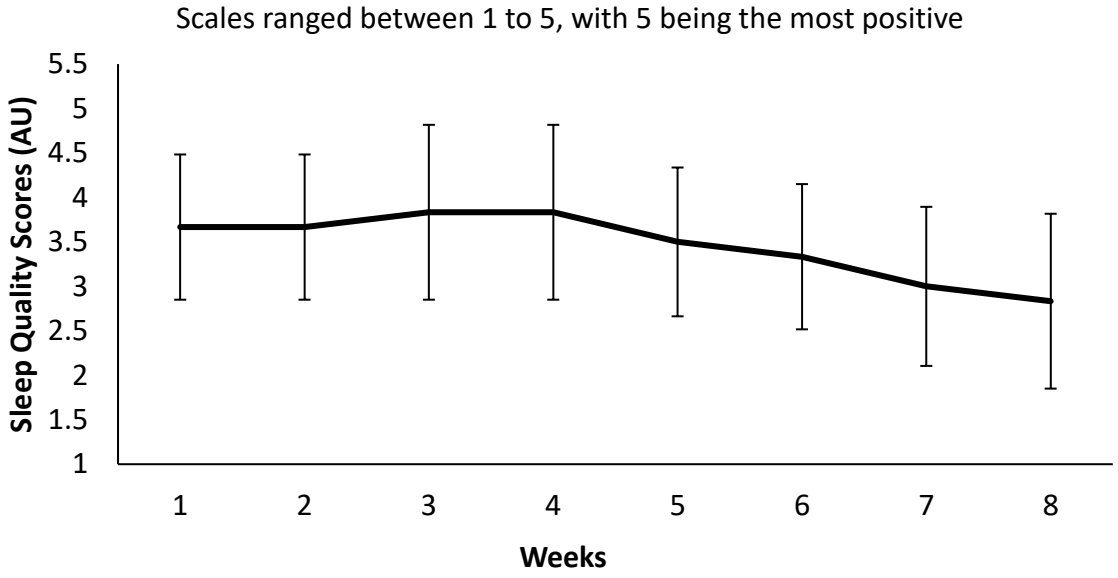

Figure 16: Weekly mean sleep quality scores (AU) across the 8-week omega-3 supplementation phase, including the SD.

No significant differences were found in the participants general muscle soreness across the 8 weeks of omega-3 supplementation. The mean general muscle soreness score was 3.2 (0.8) AU*, F*(7, 35) = 1.7, *p* = 0.1. Furthermore, no significant differences were found from week 1 to week 8 (week 1: 3.3 [1.2] vs week 8: 2.8 [0.9] AU; *F*[1, 5] = 0.4, *p* = 0.6).

A significant decrease was found in the participants mean stress level scores across the 8 weeks of omega-3 supplementation. The mean stress level score was 3.0 (0.7) AU*, F*(7, 35) = 2.3, *p* = 0.05. However, there was no significant differences from week 1 to week 8 (week 1: 3.5 [0.5] vs week 8: 2.6 [0.9] AU; *F*[1, 5] = 4.3, *p* = 0.09 [see Figure 17]).

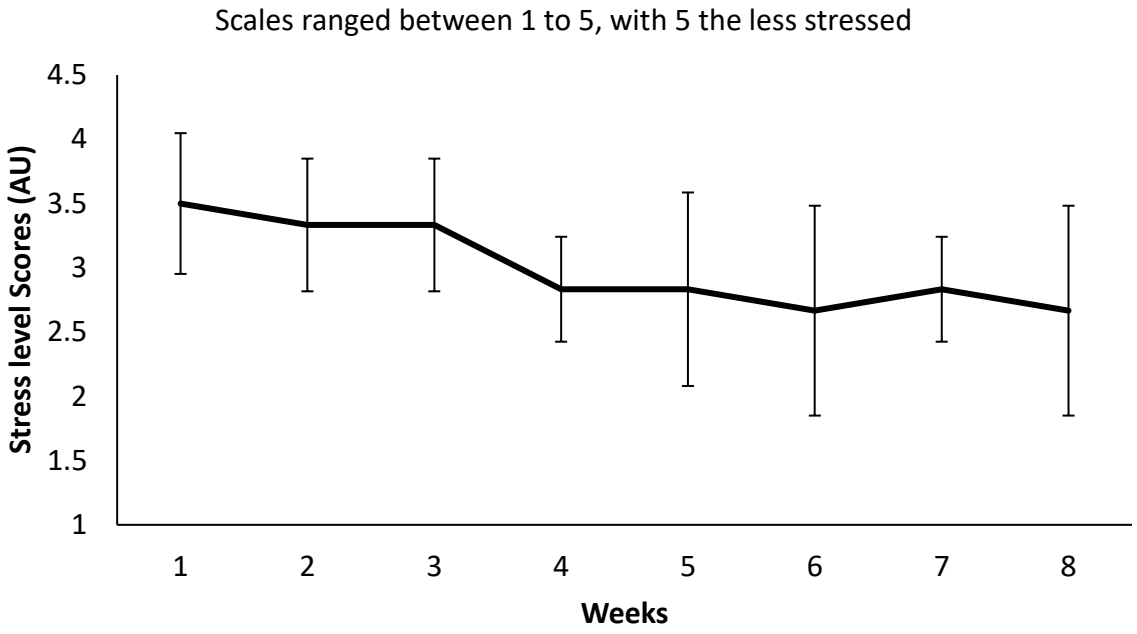

Figure 17: Weekly mean stress level scores (AU) across the 8-week omega-3 supplementation phase, including the SD.

A significant decrease was found in the participants mean mood scores across the 8 weeks of omega-3 supplementation. The mean mood score was 3.0 (0.9) AU*, F*(7, 35) = 2.4, *p* = 0.04. Furthermore, a significant decrease was found from week 1 to week 8, during the 8 (week 1: 3.5 [0.8] vs week 8: 2.7 [0.9] AU; *F*[1, 5] = 7.4, *p* = 0.04 [see Figure 18]).

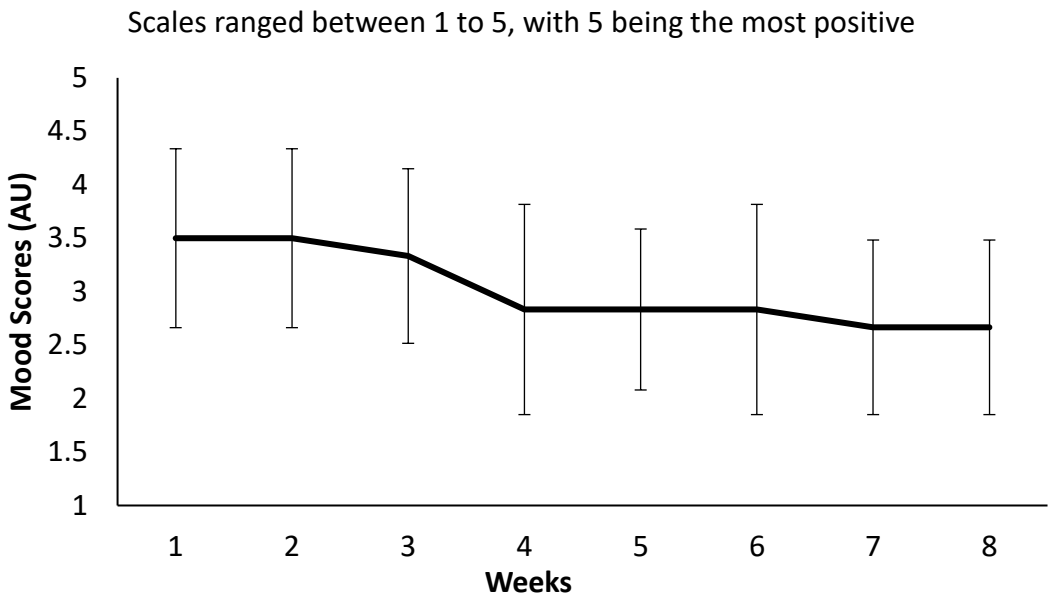

Figure 18: Weekly mean mood scores (AU) across the 8-week omega-3 supplementation phase, including the SD.

Chapter 6. Discussion

The main aim of this study was to explore the effectiveness of a 56-day (8-week) omega-3 supplementation period on the endurance capacity, performance, and neuromuscular fatigue in well-trained cyclists. This study found no significant impact of omega-3 supplementation on body composition (p = 0.3). Additionally, there were no significant differences in VO_2max, TT performances, and CMJ measures. However, given the limited previous research in these areas, further studies are needed to validate these findings. Furthermore, these cyclists adhered to a consistent training schedule during the 56-days (8-week) omega-3 supplementation period, however, they consistently failed to meet CHO and fat intake guidelines. Nevertheless, the well-being questionnaire found significant differences in certain aspects of recovery and well-being, including fatigue, stress, and mood states.

6.1 Skinfolds

The results from this study failed to show any significant differences in either body mass or mean sum of 8-site-skinfolds from pre- to post-omega-3 supplementation (p = 0.3). Furthermore, no significant differences were found in any of the eight specific skinfold sites (triceps, subscapular, bicep, lliac crest, supraspinal, abdominal, front thigh, and medical calf) pre- to post-omega-3 supplementation (p > 0.05). It is important to note that these participants did not significantly change their dietary intake (protein, CHO, fat, and/or omega-3) and duration of exercise during their supplementation phase (p > 0.05). Thus, suggesting these results were unlikely effected by variations of diet and exercise. In comparison to the studies found in the systematic review, this was the first study to compare body composition

using the 8-site skinfolds pre- to post-supplementation. As a result, these findings have contributed to addressing a gap in the literature on well-trained cyclists. However, further studies are needed to expand upon this knowledge and validate these results.

Research by Heileson and colleagues (2022), investigated the effect of 8-weeks of omega-3 (3000 mg per day) supplementations in comparison to a placebo (high-oleic safflower oil) in student athletes (n = 27) from a range of sports (track and field, baseball, sprinting, volleyball, crew, and gymnastics). The data from this study agrees with our findings in that no significant differences in body mass were found following a period of supplementation (p = 0.4). Furthermore, this study monitored the participants dietary intake pre- and during the supplementation phase, finding no changes in calorie (p = 0.9), protein (p = 0.4), CHO (p = 0.9), and fat (p = 0.6) intakes, respectively. As training habits were consistent and dietary habits were unchanged, Heileson and colleagues (2022) findings suggest that omega-3 supplementation has no significant effect upon an athlete's body mass. However, as this study did not use a cycling population within their protocol, it could not be included within the systematic review for further analysis of the study's findings.

Even though cycling is not a weight bearing sport, previous research suggests that body mass can influence performance (Impellizzeri et al., 2005; Maciejczyk et al., 2015), with Swain (1994), estimating that body mass differences can amount for ~ 10 – 20% of elite cycling performance variability. This may be due to fat mass acting as an additional load, without providing any functional benefit, thus resulting in the need for a higher generation of power (Kitagawa et al., 1980). However, in this study, no significant changes in body mass were observed with 1600 mg of omega-3 for 56-days/8-weeks. Therefore, it suggests that omega-3 supplementation does not affect an endurance cyclist's performance outcomes.

6.2 Maximal Oxygen Uptake (VO_2max)

The results from the time taken to reach volitional exhaustion during the VO_2max test found no significant differences from pre- to post-omega-3 supplementation (p = 0.2). This is supported by the findings from the systematic search (Ávila-Gandía et al., 2020; Poprzecki et al., 2009), who also found no significant differences in time to reach volitional exhaustion following a period of omega-3 supplementation (p > 0.05). Furthermore, no significant differences were found in the relative VO_2max ($ml \cdot kg \cdot min^{-1}$) values during the studies duration from pre- to post-omega-3 supplementation (p = 0.2). This is supported by the findings from the systematic search (Ávila-Gandía et al., 2020; López-Román et al., 2019; Peoples et al., 2008; Poprzecki et al., 2009), who all found no significant differences in relative VO_2max values from pre- to post-omega-3 supplementation (p > 0.05).

A systematic review (21 studies) by Fernades and colleagues (2024) investigated the effect of omega-3 supplementation in elite and amateur athletes. A total of 10 studies included a VO_2max protocol in their design (Capó et al., 2015; Delfan et al., 2015; Drobnic et al., 2021; Filaire et al., 2010; Lewis et al., 2015; Martorell et al., 2015; Raastad et al., 1997; Tomczyk et al., 2023; Żebrowska et al., 2015; Żebrowska et al., 2021). However, only one study by Żebrowska and colleagues (2015), found a significant increase in VO_2max from pre- to post-omega-3 supplementation. This study had endurance cyclists (n = 13) ingest 1300 $mg \cdot day^{-1}$ of omega-3 for 3-weeks, followed by a 2-week washout period, after which participants ingested the same supplement but without the omega-3. This study found significant differences (p < 0.05) in VO_2max values from pre- to post-supplementation in the omega-3 group (69.8 ± 4.9 to post 74.8 ± 5.6 $ml \cdot kg \cdot min^{-1}$) and in the placebo group (69.8 ± 4.9

to 71.0 ± 4.1 ml·kg·min^{-1}). These authors theorised that the significant increase in VO_2max may be due to enhanced endothelial functions. However, the placebo (gelatine) group also found a significant increase in VO_2max, suggesting that this study's findings may have been affected by other variables, such as the VO_2max protocol design. This studies design included a two-week washout period after 3-weeks of omega-3 supplementation, followed by a 3-weeks of placebo supplementation. According to Cao and colleagues (2006), omega-3 supplementation can be incorporated into cellular membranes and remain there for ~6-weeks. Therefore, suggesting that the significant increase in VO_2max observed within the placebo group, might be due to residual effects of omega-3 supplementation rather than the placebo supplementation. However, this study was not included within this systematic search as it did not meet the inclusion criteria (omega-3 supplementation > 4-weeks). As only one previous study has found a positive outcome to VO_2max from omega-3 supplementation, it still leaves a gap in the research to consolidate the findings as to whether omega-3 can lead an increase in VO_2max in an endurance cycling population.

Furthermore, the results from the maximal power achieved at VO_2max (Wmax), saw no significant differences from pre- to post- omega-3 supplementation (p = 0.3). This is supported by the findings in the systematic results (Ávila-Gandía et al., 2020; López-Román et al., 2019; Peoples et al., 2008; Poprzecki et al., 2009), as these authors also found no significant differences in maximal power produced during the VO_2max from pre- to post-omega-3 supplementation (p > 0.05). However, López-Román and colleagues (2019) found a significant decrease in maximal power output (Wmax) within their competitive cyclist group pre- to post- omega-3 supplementation (415 ± 15 vs 412 ± 35 Wmax; p = 0.02), compared to the non-competitive group which found a significant increase (355 ± 40 vs 365 ± 28 Wmax; p

= 0.05). Indicating that amateur non-competitive cyclists can benefit from omega-3 supplementation. This is supported by Christie and colleagues (2009), who determined that training status can influence VO_2max results. However, as well-trained cyclists need to efficiently transfer aerobic and anaerobic energy into power to benefit their endurance performances (Joyner & Coyle, 2008), it is important to determine if omega-3 supplementation could help increase power outputs during VO_2max testing. However, as amateur cyclists are less trained, they could have more potential to improve their power output in comparison to well-trained cyclists who have already utilised their training to reach their optimal physiological limits, making it harder to find improvements (Sandbakk & Holmberg, 2017). As the only study to date to find a significant increase in maximal power achieved at VO_2max used non-competitive cyclists (with the same protocol used on competitive cyclists), it suggests that our study, which used well-trained endurance cyclists, was unlikely to observe any significant findings.

Finally, no significant differences were found between the maximal HR produced pre- to post- omega-3 supplementation during the VO_2max test (p = 1.0). However, both Ávila-Gandía and colleagues (2020) and Peoples and colleagues (2008) found a significant decrease in maximal HR in their omega-3 groups from pre- to post-supplementation during the VO_2max test (p < 0.05). When comparing these two studies protocols, Ávila-Gandía and colleagues (2020) had an omega-3 supplementation of 1220 mg per day, for 30-days in amateur cyclists and Peoples and colleagues (2008) had an omega-3 supplementation of 3200 mg per day for 8 weeks in well-trained cyclists. These protocol designs still leave room for interpretation for future studies aiming to replicate their positive outcomes. This is due to VO_2max being an indicator of an athlete's aerobic capacity (Bassett & Howley, 2000; Ranković et al., 2010). It is

therefore important to determine if omega-3 supplementation could lead to greater cardiovascular fitness which could present as a lower maximal HR in well-trained cyclists (Barlow et al., 1985). However, as these cyclists are well documented in having a lower HR due to their greater experience with endurance training (Lucia et al., 1999; Lundstrom et al., 2023; Wundersitz et al., 2020), trying to find reductions in HR during VO_2max could again be difficult as this population group (well-trained) may have reached their optimal physiological limits (Sandbakk & Holmberg, 2017), which may be confirmed by our study findings from pre- to post-supplementation (pre: 173 [20] vs post: 173 [14] $b \cdot min^{-1}$; $F[1, 5] = 0.0$; $p = 1.0$).

6.3 Steady State Cycle (SSC)

During the SSC part of this study there were no significant differences found in the mean VO_2 ($ml \cdot kg \cdot min^{-1}$) from pre- to post-supplementation ($p = 0.1$). In comparison to the results found in the systematic review, Hingley and colleagues (2017) also found no significant differences in mean VO_2 from pre- to post-supplementation. However, this study only used a 10-min SSC. Furthermore, McAnulty and colleagues (2010) found the mean VO_2 was similar across their multiple supplementation groups (placebo 2589 ± 180, vitamin and mineral 3025 ± 153, omega-3 2725 ± 170, omega-3, vitamin and mineral 2846 ± 193 $ml \cdot kg \cdot min^{-1}$, $p = 0.6$). Therefore, these results suggest that omega-3 supplementation and/or the SSC protocol (duration) does not affect substrate utilisation in a well-trained cyclist's population, suggesting that that are not utilising their muscle glycogen storage capacities (Kiens et al., 1993).

In addition to VO_2, there were no significant differences found in the internal training load of mean HR from pre- to post-supplementation for all participants (p = 0.1). This is supported by the findings from the systematic review (Macartney et al., 2014; McAnulty et al., 2010; Nieman et al., 2009; Poprzecki et al., 2009), who also found no significant differences in maximum HR from pre- to post-omgega-3 supplementation (p > 0.05). However, as these studies examined the maximum HR and the current study examined mean HR, no direct comparisons of HR during SSC can be made, yet the rationale of quantifying maximum HR should be questioned as it is unlikely to be variable of interest given the steady state nature of this phase of the experimental design. Therefore, more research is needed to consolidate these findings. Even though, Peoples and colleagues (2008) instructed their participants to cycle to exhaustion with only the first 60- min of data being recorded, this author did not state the times produced during the SCC. Therefore, to date this was the only study to use a 75-minute SSC during an omega-3 supplementation protocol.

6.4 16.1 km Time Trial (TT)

There were no significant differences found in the time taken to complete the 16.1 km TT from pre- to post-supplementation (p = 0.1). This finding is supported by the results found in the systematic review (Da Boit et al., 2015; Lewis et al., 2015; Nieman 2009), which also found no significant differences in TT times following a period of omega-3 supplementation (p > 0.05). Suggesting that omega-3 supplementation has no effect upon TT performance times. However, due to the high variability of the TT protocols (20-min at 250 KJ [Lewis 2015], 10-min immediately at the end of a 1 h SSC [Nieman 2009], and cycling until participants

reached 70% Wmax [Da Boit et al., 2015]), utilised by these authors, future researchers need to correspond with previous protocols to allow direct comparisons.

Furthermore, no significant differences were found in the peak power achieved during the TT from pre- to post- omega-3 supplementation ($p = 0.6$). This is supported by Hingley and colleagues (2017) who also found no significant differences in power output from pre- to post-omega-3 supplementation ($p > 0.05$). This suggests that omega-3 supplementation has no effect upon peak power achieved during a TT performance. However, as no significant changes were observed, it suggests that these cyclists were able to maintain their fitness throughout the duration of the study. This is consistent with previous research which recognised that experienced endurance athletes are skilled at pacing during a TT (Abbiss et al., 2016; Atkinson & Brunskill, 2000; Micklewright et al., 2010).

Finally, no significant differences were found in the peak HR during the TT from pre- to post-supplementation ($p = 0.2$). This is supported by Da Boit and colleagues (2015), who also found no significant differences in maximum HR from pre- to post-omega-3 supplementation during the TT ($p > 0.05$), implying that omega-3 supplementation has no effect upon maximum HR achieved during a TT. Additionally, these results suggest that omega-3 supplementation does not influence fatigue levels during a well-trained cyclists TT performance, as indicated by no changes being observed in maximal HR. However, these fatigue levels could have been influenced by other factors, such as muscle energy reserves and lactate accumulation (Abbiss & Laursen, 2005; Sahlin, 2014). Therefore, to further explore omega 3's effect upon fatigue, other measures such as CMJ were used.

However, to date, no other study has investigated the effect of omega-3 supplementation on a 16.1 km TT performance, despite a 16.1 km TT being consistently

utilised by other researchers using other ergogenic aids such as dietary nitrate, blackcurrant extract, beetroot juice, sodium bicarbonate, and spirulina (Gurney et al., 2022; Lane et al., 2014; Lansley et al., 2011; Leach et al., 2013; Murphy et al., 2017).

6.5 Countermovement Jumps (CMJ)

This study found that 8-weeks of omega-3 supplementation had no significant effect on the maximal heights, flight times, and velocities produced during the CMJ's protocol from pre- to post-omega-3 supplementation ($p > 0.05$). This is supported by Lewis and colleagues (2015), who also found no significant differences in CMJ heights (cm) from pre- to post-omega-3 supplementation ($p > 0.05$). This study did not include flight times (m/s) and velocities (m/s) in their findings.

However, research by Black and colleagues (2018), who investigated if 5-weeks omega-3 and protein supplementation (1546 mg omega-3; 15 g protein) would reduce muscle soreness and the maintenance of explosive power in professional rugby union players (n = 33). Participants were asked to perform three CMJs (best score being recorded) at seven different timepoints (baseline, day-5, day-12, day-16, day-19, day-22, and day-35) throughout the supplementation period. The results from this study showed a significant increase (4.6 ± 5.9%, p = 78.5) in peak force from baseline to day 35 (2705.2 ± 271.4 vs 2816.0 ± 309.0 N) in the omega-3 group, in comparison to the placebo group (2858.5 ± 339.7 vs 2772.4 ± 481.1 N) with a significant decrease (3.4 ± 8.6%, $p > 0.05$). Furthermore, Jakeman and colleagues (2017), investigated the effect of ether a high dosage (750 mg EPA; 50 mg DHA) or low dosage (150 mg EPA; 100 mg DHA) of omega-3 supplementation (taken immediately after a muscle

damaging exercise protocol) on CMJ height in physically active males (*n* = 27). The data from this study identified that participants consuming the high omega-3 dose returned to within 2% of baseline CMJ height with 24 h, whereas the low omega-3 group only returned to within 4% of baseline CMJ height within 96 h. Thus, suggesting that a higher dose of omega-3 is likely to have a greater benefit on CMJ heigh. These findings suggest that omega-3 supplementation can positively influence an athletes NMF. However, these studies had a shorter omega-3 supplementation period (5-weeks), in comparison to our study (8-weeks [56-days]), it suggests that future research should experiment with shorter durations. However, it could be theorised that longer omega-3 supplementation periods may be more beneficial, as extended durations allow more time for the omega-3 to saturate into the muscles, potentially enhancing its effectiveness (Gerling et al., 2014). However, to date this has not been confirmed, for example a study by Heileson and colleagues (2022), investigated the impact of omega-3 supplementation on strength and power in collegiate athletes (*n* = 27). Participants were given 3000 mg $g \cdot day^{-1}$ of omega-3 for 8-weeks, and told to perform three CMJs for standardisation both pre- and post-supplementation. Finding, that CMJ height had no significant effect (p = 0.3) from pre- to post-omega-3 supplementation (42.8 ± 7.5 vs 43.5 ± 7.3 cm). As these studies did not include a cycling population, they were not included within the final systematic review results.

Furthermore, no significant differences were found found between the CMJ timepoints (pre to post SSC, pre to post TT, and post SSC to post TT) throughout the exercise protocol both pre- and post- omega-3 supplementation (p > 0.05). However, after visual inspection of figures 4 and 5, there was less statistical difference between the participants CMJ heights post-supplementation (as confirmed in Table 8). This suggests that even though

statistical analysis did not find any significant differences, there may be a trend towards a subtle effect that could only been seen in the visual data. The lack of significant results in the statistical tests (Tables 8 and 9) may be attributed to factors such as sample size, variability in individual responses, or the measurement tools used to record the CMJ data (*My Jump Lab*). This suggests that a larger sample size ($n > 6$) could potentially identify an effect of omega-3 supplementation in reducing NMF in well-trained cyclists. This is further supported by our *Post hoc* power analysis, which indicated that a study designed with 90% power to detect a medium effect size ($d = 0.5$) would require a sample size of 44, compared to the six participants in this study. Therefore, future research should aim to significantly increase the sample size to mitigate limitations in statistical power.

Even though this study did not find any significant results of NMF during CMJ after omega-3 supplementation, the findings by Black and colleagues (2018), suggest that omega-3 could have a positive effect within athletes, which could lead to well-trained cyclists utilising this during their performance, such as sprint finishes.

6.6 Food Diary

As previously indicated in Chapter 1.4, cyclists should be aiming towards 6 to 10 g per kg of body weight of CHO (Clifford & Maloney, 2015; Mata et al., 2019; Pendergast et al., 2011), 20 to 35% of your daily total energy intake as fats (Jensen et al., 2014), and 1 to 1.6 g per kg of body weight of protein (Lemon, 2000; Meredith et al., 1989). Based on the participants mean body mass (86.8 kg, post-supplementation), their daily dietary intake requirements should range between: (1) CHO = 520.8 – 868 g, (2) fat = 17.4 – 30.4 g, and (3)

protein = 86.8 – 138.9 g. Therefore, based on the food intake findings these participants failed to consume the appropriate daily CHO and fat intakes. Furthermore, as macronutrient (CHO, fat, and protein) intakes were not significantly different throughout the 8-weeks of omega-3 supplementation ($p > 0.05$), this indicates the participants were consistently not consuming the appropriate amounts of CHO and fats. This is an important finding, as researchers have provided convincing evidence that CHO and fats are essential energy sources for endurance performance (Lowery, 2004; Noakes, 2000; Pendergast et al., 1996). Therefore, this whole population group may be under-fuelling their workouts and thus compromising their exercise performance (and recovery). Additionally, it is important that well-trained cyclists consume appropriate amounts of protein so their working muscles can develop, and muscle fibres can repair the damage incurred during endurance performances (Huard et al., 2002; Stokes et al., 2018). Data from this study suggests that this population were unable to meet the suggested guidelines.

This indicates that these participants were not consistently supplying their energy systems for the research protocol and their personal training sessions, which has previously been found to effect endurance performances (Lambert & Goedecke, 2003; Sherman & Wimer, 1991). However, before this trial commenced, participants were provided with resources on specific dietary needs for their cycling training statuses. This was provided as previous research has found that athletes find it challenging to adhere to nutritional recommendations (Baranauskas et al., 2015; Hornstrom et al., 2011). Bentley and colleagues (2019) identified five barriers for adherence; (1) responsibility, (2) performance accountability, (3) role conflict, (4) environmental incongruence, and (5) stretched service. Therefore, future studies should implement more robust dietary tracking and support mechanisms to ensure

participants are meeting their nutritional needs, such as regular conversations with a nutritionist or educational interventions prior to the research study.

Finally, no significant differences were found between the weekly omega-3 (g) intakes over the 8-week omega-3 supplementation period (p = 0.6). The authors in the systematic review who utilised a food diary only compared differences in dietary habits between the omega-3 and placebo groups, finding no significant differences in omega-3 (p > 0.05). This could have influenced our studies data, as our research design was based on these previous findings. Therefore, if the previous studies did not accurately differentiate omega-3 intakes between groups, it might have led to skewed results in our study. This was the first study to monitor well-trained cyclists' dietary intakes during omega-3 supplementations, indicating the participants consistently adhered to the daily supplements and the omega-3 guidelines set by the researchers.

6.7 Training Log

During the omega-3 supplementation period, no significant differences were found in days of activity per week (p = 0.9), duration of (p = 0.5), and mean HR during these activities (p = 0.1). Indicating that well trained cyclists did keep to a consistent exercise routine throughout the 8-weeks omega-3 supplementation period. In comparison to the studies found in the systematic review, this was the first study to monitor well-trained cyclists' training routines during an omega-3 supplementations period. However, these findings are supported by research of Hecksteden and colleagues (2018), who determined that most training studies that range between 4 to 12 weeks, are a feasible duration for participants to

keep a consistent training routine. By the participants keeping to a consistent training routine, it reduces the possibility of any performance changes (e.g. increased muscular fatigue, improvements in VO_2max), which may be impacted by variations in training volumes or intensities. Furthermore, overtraining can lead to a decrease in muscular strength, which is essential for endurance athletes (Faria et al., 2005; Snyder, 1998). Furthermore, overtraining can lead to an increased resting HR, which could indicate glycogen depletion (Currell et al., 2006; Stone et al., 1991). Undertraining can increase the risk of injury when returning to exercise performances (Gleeson, 2002). Therefore, by participants maintaining a consistent training routine this can be considered important in isolating the effects of omega-3 supplementation on performance outcomes by reducing confounding variables caused by over- and/or under-training.

6.8 Athlete Wellbeing Questionnaire

This study found a significant decrease in the participants perceived fatigue, sleep quality, stress levels, and mood during the 8-week omega-3 supplementation phase ($p < 0.05$). However, the participants general muscle soreness found no significant differences ($p = 0.1$), which tends to support our earlier data indicating NMF was not compromised, and participants maintained a consistent training pattern. In comparison to the studies found in the systematic review, this was the first study to monitor the cyclist's wellbeing during the supplementation phase and therefore, no direct comparisons can be made.

Poor perceived fatigue can significantly impact a cyclist's performance by reducing physical and cognitive functions, such as decision making and reaction times, which can

increase the risk of injury (Edwards et al., 2018, 2021; Taylor et al., 2012). Furthermore, sleep is essential for recovery and muscle repairs, therefore limited sleep can negatively affect a cyclist's strength endurance and cognitive functions (Kirschen et al., 2020; Knowles et al., 2018). Stress can also hinder a cyclists recovery process by increasing noradrenaline, which can result in increased overall HR (Faria et al., 2005; Hooper et al., 1999). Mood can be used as a predicter of performance, as some athletes consult their emotions on whether they have the psychological resources necessary for a successful performance outcome (Lane et al., 2004; Lane et al., 2005). Therefore, these findings suggest that omega-3 supplementation positively affects a well-trained cyclists perceived fatigue, stress levels, and mood. However, omega-3 appears to decrease a cyclists sleep quality and has no effect upon general muscle soreness.

Research by Black and colleges (2018), investigated 20 professional rugby union players perceived fatigue, sleep, stress, and mood each morning of training for 5 weeks of 1546 mg omega-3 and 15 g protein supplementation. This questionnaire was based upon a previous study by Mclean and colleagues (2010), with participants rating their responses using a 5-point Likert scale (with higher responses corresponds to positive results). Finding a likely moderate beneficial effect upon fatigue between days 20 – 35 ($p < 0.05$), however there was only a trivial or small unclear effect upon sleep, stress, and mood throughout the 5-week period. These results suggest that omega-3 (with protein) supplementation can help reduce fatigue following at least 2 weeks of supplementation. Additionally, suggesting omega-3 and protein has a minimal effect upon an athlete's sleep, stress, and mood. As this was one of the few studies to examine sleep quality from omega-3 supplementation in athletes, the findings suggest that omega-3 has a limited role in improving sleep. However, Fontani and colleagues

(2005), investigated the cognitive and physiological effects of omega-3 supplementation (4000 mg per day for 35 days) in 33 healthy active participants using a profile of mood states test, finding that the participants mood profiles had decreased in anger ($p < 0.001$), anxiety ($p < 0.01$), and depression ($p < 0.01$) states after omega-3 supplementation phase. Even though, these studies did not include a cycling population (and therefore was not included within the systematic review), there findings support that omega-3 can benefit an athlete's fatigue, and mood states.

A study by VanDusseldorp and colleagues (2020) investigated perceived muscle soreness scores following a muscle damage exercise protocol (1 hr, 2 hrs, 4 hrs, 24 hrs, 48 hrs, and 72 hrs) in 32 college athletes after 7 weeks of ether 2, 4, or 6 g of omega-3 supplementation per day. Finding that 6 g of omega-3 per day displayed lower perceived muscle soreness scores post-exercise ($p < 0.05$), in comparison to the other omega-3 groups ($p > 0.05$). These findings indicate that higher intakes of omega-3 (6 g per day) may be necessary for athletes to achieve a reduction in general muscle soreness. However, recommendation conflicts with the EFSA (2012) guidelines, which state that omega-3 is safe to consume up to the maximum of 5,000 mg (5 g) per day.

These findings suggest that 8 weeks of 1600 mg of omega-3 daily supplementation can enhance certain aspects of recovery and mental well-being, such as fatigue, stress levels, and mood states, in well-trained endurance cyclists. However, further research is needed to determine is sleep quality could improve following omega-3 supplementation and if general muscle soreness can improve form higher dosages of omega-3 in this population, as long as it corresponds with EFSA (2012) guidelines.

Chapter 7. Conclusion

In conclusion, this study found that supplementing 1600 $mg \cdot day^{-1}$ of omega-3 for 56-days (8-weeks), did not have a significant impact on sum of skinfolds (8 sites), VO_2max, SSC and TT performances in well-trained endurance cyclists. Indicating that omega-3 supplementation has no significant effect upon the endurance capacities, performances, and NMF in well-trained cyclists. However, this study highlighted that these athletes may not meeting their CHO and fat intake needs, which may have hindered their endurance performance(s). Additionally, omega-3 supplementation did show significant benefits in enhancing certain aspects of recovery and well-being in these cyclists, as indicated in no significant differences found in CMJ heights from pre- to post-supplementation (p = 0.6).

Despite the lack of significant findings, this research does add to the existing knowledge on the optimal dosage and duration of omega-3 supplementation for improving the performance and NMF of well-trained endurance cyclists.

7.1 Limitations

A limitation found from this research were the cyclists not consistently consuming enough macronutrients (CHO and fats) throughout the duration of the trial. Therefore, future studies should implement more robust dietary tracking and support mechanisms, such as regular conversations with a nutritionist or educational interventions, to help cyclists understand the crucial role that dietary intake plays in influencing endurance performances. Another limitation includes the lack of participants who partook in this study (n = 6), with (n

= 3) withdrawing. As larger sample sizes can provide a better represent the chosen population, creating a more reliable result outcome due to the smaller margins of error (Andrade, 2020). Additionally, a larger sample size could have increased the likelihood of detecting significant results by enhancing the statistical power and improving the ability to identify any meaningful effects of omega-3 on endurance, NMF, and recovery in well-trained cyclists. This is supported by our *Post hoc* power analysis, which confirmed that our study was significantly underpowered (*n* = 6 vs. *n* = 44) to detect a medium effect size. Future research should consider a substantially larger sample size to improve the reliability of findings while being mindful of time constraints and participant burden (e.g., training and food logs, daily ingestion of omega-3 beverage) as previously described (Lakens, 2022).

7.2 Future Directions

Future research may wish to investigate the effects of varying dosages and durations of omega-3 supplementation to better understand its impact on VO_2max. With smaller dosages of 1300 $mg \cdot day^{-1}$ for 3-weeks, as suggested by Żebrowska and colleagues (2015), to further explore the potential positive outcomes on VO_2max. It would also be beneficial to explore larger dosages (>1300 mg) of omega-3 over short and extended durations (≥ 3-weeks) of supplementation. By comparing these dosages and durations, it could provide a greater insight into how omega-3's might enhance endurance performances in well-trained cyclists.

Due to the limit number of studies on omega-3 supplementation in endurance cyclists, further research is needed to draw more definitive conclusions. They should use more consistent and standardised protocols to enable more direct comparisons across research

findings. For example, research should focus on incorporating more specific blood markers relating to muscle damage. By monitoring these biomarkers, it can provide further insights into how omega-3 supplementation can influence muscle recovery and inflammatory responses. Therefore, this would contribute to a greater understanding of how to enhance recovery and endurance performance in well-trained cyclists. Finally, future studies should consider conducting and reporting an *a priori* power calculation, as recommended by Abt et al., (2020) and Lakens (2022), to improve sample size estimations, enhance scientific transparency, and strengthen the robustness and reproducibility of findings.

Chapter 8. References

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Chapter 9. Appendices

A. FHS REC Form

RESEARCH ETHICS COMMITTEE

FORM A – New Application

(Involving human participants, subjects or material)

It is essential that you are familiar with the University Code of Good Research Practice, Research Ethics Policy and the Procedures for Granting Ethical Approval before you complete this form that can be found here. Please confirm that you have read and understood these documents:

X	Yes		No

Please read each question carefully, taking note of instructions and completing all parts. If a question is not applicable please indicate so. Where a question asks for information which you have previously provided in answer to another question, please refer to your earlier answer rather than repeating information.

Ethics reference number (for office use):	
WorkTribe project URL	

PART A: SUMMARY
A.1 Title of the research Effectiveness of omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation on endurance capacity, performance, and neuromuscular fatigue in cyclists.

A.2 Principal investigator's contact details	
Name *(Title, first name, surname)*	Miss Abbie Healy
Position	Student
Faculty/School	Sport, Exercise and Rehabilitation Sciences
Telephone number	
University of Hull email address	a.healy-2018@hull.ac.uk
A.3 To be completed by students only	
Qualification working towards (e.g. Masters, PhD, ClinPsyD)	Masters by Research
Student number	201908985
Supervisor's name (Title, first name, surname)	Dr James Bray
Faculty/ School	Sport, Exercise and Rehabilitation Sciences
Supervisor's telephone number	
Supervisor's email address	j.bray@hull.ac.uk
A.4 Other relevant members of the research team (e.g. co-investigators, co-supervisors)	
Name *(Title, first name, surname)*	Dr Rebecca Vince
Position	Co-Supervisor
Faculty/ School	Sport, Exercise and Rehabilitation Sciences
Telephone number	
Institution	The University of Hull
Email address	Rebecca.Vince@hull.ac.uk
Name (Title, first name, surname)	Dr Leigh Madden
Position	Co-investigator
Faculty/ School	Faculty of Health Sciences
Telephone number	
Email address	l.a.madden@hull.ac.uk

	Research involving discussion of sensitive topics or topics that could be considered sensitive
	Research involving discussion of culturally sensitive issues
X	Prolonged or frequent participant involvement
X	Research involving members of the public in a research capacity (participant research)
	Research conducted outside the UK
	Research involving accessing social media sites
	Research involving accessing or encountering security sensitive material
	Research involving accessing websites or material associated with extreme or terrorist communities
	Research involving storing or transmitting any material that could be interpreted as sympathetic, endorsing or promoting terrorist acts
	Research involving financial inducements for participants (other than reasonable expenses and compensation for time)

A.6 If you are an employee of the university, are you employed under an academic contract? ***(applicable to University staff only)***

	Yes		No

If not, please explain very briefly why the research is required / permitted and provide evidence of permission to conduct the research from your line manager and any other appropriate party.

A.7 Will this study be pre-registered with an online registry (such as OSF.org.io, or AsPredicted.org)

	Yes	X	No

If yes, please give the name of the registry. If the study has already been pre-registered, you should also provide the URL and/or study ID.

A.8 Will this study be considered by any Ethics Committees external to the Faculty of Health Sciences?

	Yes	X	No

If yes, please identity the external committee:

PART B: THE RESEARCH

B.1 Give a short summary of the research (max 300 words)

This study aim is to investigate the effectiveness of a commercially available omega-3 and protein sport nutrition beverage on endurance performance, neuromuscular fatigue, and recovery in well-trained cyclists.

This is important for endurance athletes as they continually seek to enhance their exercise capacity and substrate utilisation, whilst accelerating their recovery from exercise (Hodgson, 1985). Omega-3 can reduce exercise-induced inflammation, improve skeletal muscle function, and energy availability in athletes (Simopoulos, 2007). Furthermore, prolonged omega-3 intake is suggested to alter ATP synthesis by increasing the capacity for mitochondrial reactive oxygen species emission without altering the content of oxidative products (Herbst et al., 2014; Baker, McCormick & Robergs, 2010). Suggesting that enhancing the oxidation of fatty acids, can improve an endurance athlete's ability to utilise substrates and maximise available stores of muscle glycogen.

There will be a series of four laboratory visits; visit one, will consist of familiarisation, including, anthropometrical measures and a maximal oxygen uptake (VO2max) test, using a 50 watt (W), ramp protocol on a cycle ergometer. Metabolic variables (i.e., VO_2, VCO_2) will be collected through computer-assisted indirect calorimetry. Visit 2; will combine steady state cycling (75 mins at 60% VO_2 max) with a 16.1 km time trial (TT). Between visit 2 and 3, participants will consume an omega-3 and protein beverage (1600 mg omega-3 + 20 g protein) daily for 56-days

whilst following a low omega-3 diet. At visit 3, participants will follow the same procedures as visit 2. Pre-, post-, and 24 hours post TT, venous bloods will be taken (20 ml). During the visit 2 and visit 3 trials participants will also be required to complete a countermovement jump protocol (3 x maximal jumps) on a portable jump mat for an indirect measure of neuromuscular fatigue at three time points (post warm-up, post steady state cycling and post TT). Some of the blood markers of interest include inflammatory (e.g Interleukin-6 (IL-6) and interlekin-1 beta (IL-1b)), cardiovascular risk (e.g. c-reactive protein (CRP), angiogenic (e.g. vascular endothelial growth factor (VEGF), creatine kinase (CK), for signs of muscle damage, oxidative stress markers, cellular adhesion molecules and circulating microparticle population analysis. Finally, the fourth laboratory visit will be a repeat of the first laboratory visit to identify any observed changes in body composition and VO_2 max.

References:

Baker, J. S., McCormick, M. C., & Robergs, R. A. (2010). Interaction among skeletal muscle metabolic energy systems during intense exercise. *Journal of Nutrition and Metabolism*, 2010.

Herbst, E. A., Paglialunga, S., Gerling, C., Whitfield, J., Mukai, K., Chabowski, A., Heigenhauser, G. J., Spriet, L. L., & Holloway, G. P. (2014). Omega-3 supplementation alters mitochondrial membrane composition and respiration kinetics in human skeletal muscle. *The Journal of Physiology*, 592(6), 1341–1352. https://doi.org/10.1113/jphysiol.2013.267336

Hodgson, D. R. (1985). Energy considerations during exercise. *Veterinary Clinics of North America: Equine Practice*, 1(3), 447-460.

Simopoulos, A. P. (2007). Omega-3 fatty acids and athletics. *Current Sports Medicine Reports*, 6(4), 230-236.

B.2 Proposed study dates and duration

Research start date (DD/MM/YY): 06/03/23 Research end date (DD/MM/YY): 23/09/23

Fieldwork start date (DD/MM/YY): _____ Fieldwork end date (DD/MM/YY): _____

B.3 Where will the research be undertaken? (i.e. in the street, on University of Hull premises, in schools, on-line etc.)

The University of Hull premises, Exercise Physiology laboratories

Do you have permission to conduct the research on the premises?

X	Yes		No

If no, please describe how this will be addressed.

B.4 Does the research involve any risks to the researchers themselves, or people not directly involved in the research? *E.g. lone working*

X	Yes		No

If yes, please describe and say how these will be addressed (include reference to relevant lone working policies): _____

If yes, please include a copy of your completed risk assessment form with your application.

There is a small risk to the research of slips, trips, falls, and musculoskeletal injury during the administration of testing and any demonstration of exercises for this project. These are all addressed in the attached Risk Assessment Form.

During the venous blood sampling and subsequent biochemical analysis of blood samples there is a risk of spillage or cross contamination between the subject and administrator. To control for this, all spillages will be cleaned in accordance with the departmental COSHH regulations. All procedures will be completed by appropriately trained individuals wearing the relevant personal protective equipment (PPE) and in line with the relevant departmental guidelines (e.g., SOPs).

Researchers will follow all risk assessment evaluations. The researchers have had suitable training to conduct the research.

Availability of all the required personal protective equipment will be ensured and all equipment will be checked before testing to ensure it is in good working condition.

NB: If you are unsure whether a risk assessment is required visit the Health and Safety SharePoint site. Risk assessments are required for all fieldwork taking place off campus.

B.5 What are the main ethical issues with the research and how will these be addressed?

Indicate any issues on which you would welcome advice from the ethics committee

The main ethical issues with this research fall into the following categories:

- Research involving human participants
- Working with biological samples
- Supplementation protocols
- Risk associated with COVID-19
- Risk of injury associated with exercise participation
- Data handling
- Confidentially
- Anonymity
- Informed consent
- Participant information

Infection, blood spillage and sampling processing: Researchers will follow all standard operating procedures (SOPs) and risk assessments, wearing appropriate PPE (e.g., gloves, goggles, lab coat) to protect against infection, especially during blood sampling collection and use of the metabolic gas collection system (cortex). The researcher has had suitable training to conduct the research. The elements of risk will be addressed through the use of pre-test medical screening, strict exclusion criteria and a complete risk assessment. The researcher has had suitable training to conduct the research, the subjects will be closely monitored at all times and a first aider will be present at all times. During the venous blood sampling and subsequent biochemical analysis of blood samples there is a risk of spillage or cross contamination between the subject and administrator. To control for this, all spillages will be cleaned in accordance with the department COSHH regulations and samples processed according to manufactures instructions and well-established procedures. Researchers will follow all risk assessment evaluations and associated control measures have been undertaken for all aspects of the project to ensure risk is minimised. All aspects of this research have been appropriately risk assessed (please see Risk Assessment Form).

Consent: To ensure all participants are fully informed about this research study, participants will be provided with a participant information sheet and be required to provide written informed consent before completing the trials (found in appendix C and D).

Data Handling: All data will be kept on the student investigator's/research team's password protected computers. Data will only be shared amongst the research team. No other external parties will have access to this information. All data will be deleted after 5 years post completion of the trial.

Coercion to feel pressured or obligated to participate in the research trial: Participants will be provided with the participant information sheet and will be required to provide consent before participating. It is the participant's choice whether to participate in the research project or not. During the consent process, participants will be advised that they can withdraw from the study at any point without providing any reason for doing so and without any adverse implications.

Confidentiality and Anonymity of the participant: No identifiable information shall be collected as part of this study. Participants will answer some simple questions about the supplement and their supplement practices. Responses to the surveys will be collected electronically and data will be exported from JISC online surveys and stored on an encrypted external hard drive password protected computer or university approved cloud storage system. All information gathered in this study will be stored in line with the 2018 Data Protection Act and will be destroyed 5 years after the conclusion of this study. Access to the research data is strictly limited to the research team. All reasonable steps will be taken to ensure that confidential details are secure – data will be coded and kept on a password protected computers.

Exercise participation: this research will involve participation on supervision exercise (steady state [60% VO_2max] and performance [16.1km TT & 3 x CMJs]) in the lab at a relatively high intensity. Exercise participation does carry a degree of risk. However, participants will be accustomed to this nature of activity in both training and competition. When exercising in the lab there will also be a qualified first aider on hand at all times with access to appropriate first aid equipment. Where exercise testing is to be conducted, we will adhere to relevant governing body testing and safety protocols at all times (e.g. British Association of Sport and Exercise Sciences etc.). Additional COVID-19 measures will also be put in place as required (e.g., researcher to wear appropriate PPE and

adhere to social distancing where possible, hand washing and sanitisation of equipment after use).

To address the above issues the research will adhere to the ethical considerations outlined in the most recent revision of the Declaration of Helsinki. The benefits associated with the research are deemed to be significant enough to make the study worthwhile, participants will be given a Participant Information Sheet (see appendices below) detailing their exact involvement within the research, so they are aware of the length of time they will be involved in the research, the benefits and potential risks. In this way true and complete informed consent can be obtained.

Together with the Informed Consent Form and the Participation Information Sheet (see Appendices), participants will be made aware of how their information and data will be dealt with prior to giving consent. Participants will be anonymised and given a participation code on any paper or electronic documentation associated with the research. This is to ensure that their data is not identifiable. Data will be stored as paper copies in a locked cabinet in a secure location on the university campus, accessibility will be restricted to the research team only. Moreover, any personal data will be stored electronically on password encrypted university cloud-based software. Participants will also be made aware that they are free to withdraw their participation from the research at any point, without having to give a reason, by contacting the researcher. At this point all related data will also be withdrawn from the research study if they wish and be destroyed.

B.6 Does the research involve an international collaborator or research conducted overseas:

	Yes	X	No

If yes, describe any ethical review procedures that you will need to comply with in that country:

Describe the measures you have taken to comply with these:

Include copies of any ethical approval letters/ certificates with your application.

PART C: HUMAN PARTCIPANTS AND SUBJECTS

C.1 Are the participants expected to be from any of the following groups? (Mark with X as appropriate)

	Children under 16 years old. ***Specify age group:*** _____
	Adults with learning disabilities
	Adults with other forms of mental incapacity or mental illness
	Adults in emergency situations
	Prisoners or young offenders
	Those who could be considered to have a particularly dependent relationship with the investigator, e.g. members of staff, students
	Other vulnerable groups
X	No participants from any of the above groups

Include in Section D5 details of extra steps taken to assure their protection.

Does your research require you to have a DBS check?

	Yes	X	No

It is the researcher's responsibility to check whether a DBS check (or equivalent) is required and to obtain one if it is needed. See also http://www.homeoffice.gov.uk/agencies-public-bodies/dbs

C.2 What are the potential benefits and/ or risks for research participants in both the short and medium-term?

Benefits:

Participants will receive exercising testing and feedback post trial based on their performance during baseline testing. They will also receive a supplementation protocol that may improve their performance and offer some insight into their nutritional evaluation.

Risks may include health and safety, physical harm and emotional well-being

Minor gastro-intestinal discomfort such as slight bowel discomfort and/or feelings of nausea and bloating may be associated with the consumption of the omega-3 and protein beverage, however instances of this are highly unlikely. In rare cases, there is a possibility of allergy or overdose should the right protocol not be observed when using the supplement although instances of this are highly unlikely. Furthermore, strenuous exercise has the potential to cause musculoskeletal injury, fainting, nausea, vomiting, trip/fall and in extreme cases cardiorespiratory complications, however, instances of this are rare. There is a minor discomfort caused by the puncturing of the skin during venous blood sampling which in turn could present a risk of infection to the subject and/or cross contamination between subject and administrator. There is also a risk of feinting/falling. There is a possibility of some light bruising to the arm following venous blood draws. Venous blood sampling will be performed by either somebody who has completed the department venepuncture training or a trained medical professional. All department guidelines regarding venous blood sampling and the control of infection will be adhered to.

What will be done to avoid or minimise the risks?

The elements of risk will be addressed using pre-test medical screening, a strict inclusion/exclusion criteria and a complete risk assessment.

The actual session will be based around the athletes current training schedule and involve activities that they are aware of and therefore accustomed to. As such, the athletes will not be over-exerted and will be familiar with what is taking place. To avoid an allergy or overdose, the bespoke beverage is pre-mixed and in a single-service packet. Participants will be instructed to only consume ONE per day and will be required to confirm their have no allergies to any of the ingredients. Moreover, this product is a batch tested and complies with informed sport (https://sport.wetestyoutrust.com/supplement-search/brand/eo3enhanced-recovery), which provides athletes and researchers with a level of quality assurance.

The participants may be at risk of embarrassment when providing body weight and skinfold measures, therefore each athlete will be able to do this in private. The researcher has suitable experience and training to conduct the research, the subjects will be closely monitored at all times and a first aider will be present at all times. Also, administrators will

be wearing appropriate clothing (e.g. gloves, goggles, lab coat) to protect against infection especially during blood sampling.

All participants will be further screened for contraindications to exercise prior to taking part by completing a medical health questionnaire. If any substantial risk factors are present (e.g., prior myocardial infarction or heart failure, history of unstable angina or unstable coronary heart disease, history of chronic obstructive pulmonary disease, recent infection or fever, pre-exercising severe physical disability, pregnancy, pre-existing musculoskeletal injury or orthopaedic difficulties, COVID-19 symptoms) they will be excluded from participating. Participants will perform a warm-up and cool-down encompassing exercise to minimise risk of injury. Participants will be reminded that they are able to terminate the exercise at any point.

Social distancing measures (where possible), personal protective equipment (PPE) where appropriate, hand washing, and equipment sanitisation will be reinforced during all face-to-face contact with participants. All PPE will be available to use in the sports science laboratories. Reusable items will be sanitised after use.

C.3 Is there a potential for criminal or other disclosures to the researcher requiring action to take place during the research? (e.g. during interviews/group discussions, or use of screen tests for drugs?)

	Yes		X	No

If yes, please describe and say how these will be addressed:

C.4 What will participants be asked to do in the study? *(e.g. number of visits, time involved, travel required, interviews)*

This study will use well-trained cyclists from the local area who are not currently taking any supplements containing omega 3. Participants will be asked to travel the University of Hull's Exercise Physiology research lab to perform four trials (as detailed above [see B.1]). The visits will consist of; 1) familiarisation, anthropometrical measures and an incremental cycling test to exhaustion (VO_2 max). Trials two and three will consist of a 75-minute steady state cycle (60% VO_2max), followed by a 16.1km TT on the cycle ergometer, with an indirect assessment of NMF at three time points (post warm-up, post steady state & post TT). The first two sessions will be split by a week of each other; however, the third trial will be 56 days or 8 weeks after the second trial to allow supplementation (1600 mg omega-3 FA/day). During this time, participants will be asked to complete a food diary (only two days a week) to track omega-3 consumption in their

diet (see appendix). The fourth and final trial visit will be a repeat of the procedures completed in visit 1, to quantify test-re-test validity and observe any meaningful changes in body composition and VO_2max.

Visit 1:

Participants will meet the researchers at the University of Hull. Comfortable sports clothing and sports shoes will be required. Participants will be encouraged to attend the session in a well-hydrated state, they will be asked to refrain from any strenuous activity for 48 hours before the visit. Regular hand sanitisation will be completed throughout the session and in the various locations in which the testing will take place. The researcher will brief participants on how the session is going to run and answer any questions or concerns that they might have. If they still meet the inclusion criteria for the study following completion of the pre-exercise medical questionnaire, and confirming they have no known allergies or intolerances of the supplement, then their resting heart rate, weight, height, and body composition will be recorded. Subsequently, participants will be asked to complete an incremental cycling test to exhaustion (VO2max). This will involve participants undertaking a 10-minute warm up with a preferred workload < 150Watts (W) on the cycle ergometer. After the warm up period, the VO2max protocol will commence, and will increase 50W every minute until volitional exhaustion. Peak oxygen consumption, peak workload, submaximal and peak heart rate and participant rating of perceived exertion (RPE) will be assessed. During this test, the aforementioned data will be collected automatically by the metabolic gas-cart. Participants will be allowed to cycle at any pace 70-120 cadence rpm. These measures (skinfolds and VO2max) will be repeated after the 56-day supplementary period (visit 4).

Visit 1 should not take any longer than two hours.

Visit 2:

Prior to the trial, participants will be asked to refrain from high intensity cycling for 48 hours. They will also be instructed to arrive at the laboratory on each testing day in a euhydrated state (20 mL of fluid/kg of body weight) and fed (2 g of carbohydrates/kg of body weight), abstaining from any sources of caffeine.

When the participants arrive, the participant's ergometer will be set to their specifications so they will feel comfortable during the trial (e.g., adjusting the saddle and handlebars). Workload for the submaximal exercise component will be set at 60% VO_2max derived from the maximal oxygen consumption test. This same workload will be used after the supplementation trial, allowing for direct comparisons to be made for pre- to post- supplementation of the metabolic variables (i.e., VO_2, VCO_2) collected through computer-assisted indirect calorimetry, HR and RPE, respectively. Subjects will be allowed to consume water ad libitum throughout the tests. After the 75-minute submaximal exercise period, participants then complete the 16.1 km time trial (TT). Following the warm-up and prior to the steady state element an indirect assessment of NMF will be conducted via three maximal CMJs as well as post steady state and post TT. All participants will be asked to report their RPE after every 2 km according to the Borg scale. Furthermore, during the trial their heart rate will be monitored and recorded every 2 km. Throughout the TT, participants will be withheld from any performance information, except for distance covered.

Participants will only be informed of their performance information once the trial has been completed. Time, speed, and power output will be recorded at the end of the session by the researchers using the specific ergometer software. Venous bloods will be taken from the participants pre-, post-, and 24 hours post TT. During the trial period participants will be asked to complete a wellness (including URTI symptoms) and muscle soreness questionnaires, weekly.

It is expected each trial will not last longer than three hours.

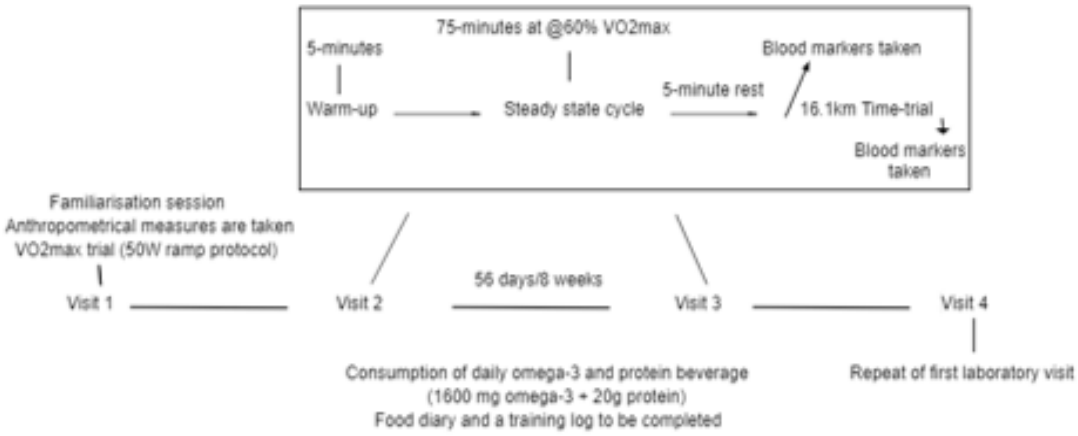

Figure 1. Study design protocol and outline

Between visit 2 and 3:

The participants will be asked for the duration of the study, to maintain a normal level of training, with the exception abstaining from high intensity cycling for 48 hours prior to testing. Furthermore, participants will be asked to refrain from consuming >3 servings of fish per week, including any omega-3 supplements not supplied by the trial's supervisors. This will be monitored by the participants keeping a food diary at fixed periods throughout the trial. For 56 days/8 weeks, participants will consume a commercially available omega-3 and protein beverage (1600 mg omega-3 + 20g protein) daily. Furthermore, participants will be asked to complete a training log to allow for weekly training load to be calculated (duration *HR).

Visit 3:

Again, participants will be asked to refrain from training high intensity cycling for 48 hours before the trial. All procedures will be identical to visit 2.

Visit 4:

As detailed previously, measures of skinfolds and re-test of VO_2max will be repeated allowing for direct comparisons to be made for pre- to post-supplementation of sum of skinfolds, peak power, metabolic variables (i.e., O_2 consumption), HR and RPE, respectively.

Blood Sampling:

Venous bloods will be taken a total of three times per experimental trial at; baseline, post, and 24-hours post exercise. Venous blood samples will be assessed for markers of cellular stress, injury and recovery (e.g. Interleukins for signs of inflammation, creatine kinase (CK) for signs of muscle damage, oxidative stress markers (e.g. TBARS, glutathione), cardiovascular risk and angiogenic markers (e.g. CRP, VEGF), cellular adhesion molecules and circulating microparticle population analysis (e.g. CD105, CD106, CD54, CD142, E-selection, P-selection) alongside angiogenic, cardiovascular risk, pro- and anti-inflammatory biomarkers and those involved in tissue repair in *in vitro* models. Clotting time and erythrocyte membrane fatty acids profile will also be assessed.

A resting blood sample (not more than 20 ml) from the antecubital vein will be collected into potassium EDTA, tri-sodium citrate coagulation and serum separator Vacutainer tubes during visits 2 and 3. Samples will be collected, process and stored at –80 degrees for later analysis of biomarkers stated above.

PART D: RECRUITMENT & CONSENT PROCESSES

How participants are recruited is important to ensure that they are not induced or coerced into participation. The way participants are identified may have a bearing on whether the results can be generalised. Explain each point and give details for subgroups separately if appropriate. Also say who will identify, approach and recruit participants. Remember to include all advertising material (posters, emails etc) as part of your application.

D.1 Describe how potential participants in the study be identified, approached and recruited and who will do this:

(i) identified:

Individuals who are well-trained cyclists will be invited to take part in the trial. Participants must be aged 18> years, and we estimate the age group of the participants will be between 20-40 years old. Inclusion criteria for the study is that participants must train for > 4 hours per week and be accustomed to high intensity exercise. They must be apparently healthy, no history of heart conditions, and free from any existing medical conditions/injuries at the time of testing; and have completed the pre-exercise medical questionnaire satisfactorily with no problems or contraindications to exercise highlighted. This will all be highlighted on all advertising material (appendix A and B). Participants must not be pregnant or trying to become pregnant and have no pre-excising musculoskeletal injuries or orthopaedic difficulties that could influence their ability to perform exercise. Participants must have no symptoms of COVID-19 and no known

allergies or intolerances to any ingredient in the supplement drink. They must be able to understand and communicate effectively in English and capable of giving written informed consent.

(ii) approached:

Various recruitment strategies will include posters around the local area (University of Hull) and different online social media platforms, such as Facebook to try and interest our target participants. Initially prospective participants will be approached by a member of the research team, via email, in person, or on the phone. They will be given the participant information sheet (appendix C) and will be given the opportunity to ask any questions.

(iii) recruited:

Participants will be given the researcher's contact details, so they can express their interest in being involved with the project. The researcher will in person/over the phone/via email explain in more detail what the research involves and ascertain that the individual meets the inclusion criteria. Following this, participants will be sent the participant within the research and can then decide whether to participate or not and must contact investigator with their decision. A member of the research team will seek the participant's informed consent (appendix D).

D.2 Will you be excluding any groups of people, and if so what is the rationale for that?

Excluding certain groups of people, intentionally or unintentionally may be unethical in some circumstances. It may be wholly appropriate to exclude groups of people in other cases

Exclusion criteria will include not meeting the requirements from the inclusion criteria. Furthermore, the participants must have no known allergy or intolerance to omega-3 or known adverse event to omega-3 supplementation and must not be currently taking any ergogenic aid or diet naturally high in omega-3s. They must also not be vegan. Furthermore, they must be able to understand the purpose of the trial and their involvement within it. They must not be under the age of 18 or unable to provide informed consent for this trial.

D.3 How many participants will be recruited and how was the number decided upon?

It is important to ensure that enough participants are recruited to be able to answer the aims of the research. The number of participants should be sufficient to achieve worthwhile results but

should not be so high as to involve unnecessary recruitment and burdens for participants. This is especially pertinent in research which involves an element of risk. Describe here how many participants will be recruited, and whether this will be enough to answer the research question.

We aim to recruit at least 10 participants. Participant numbers are based on equipment/supplement availability and the time availability to the research team in which to conduct the project.

If you have a formal power calculation, please replicate it here.

D.4 Will the research involve any element of deception?

	Yes		X	No

If yes, please describe why this is necessary and whether participants will be informed at the end of the study.

D.5 Will informed consent be obtained from the research participants?

X	Yes			No

If yes, give details of how it will be done. Give details of any particular steps to provide information (in addition to a written information sheet) e.g. videos, interactive material. If you are not going to be obtaining informed consent you will need to justify this.

Before any tests have begun, a full explanation of all aspects of the trials and procedures will be detailed in writing and verbally by talking through the participant information sheet. They will also be made aware of their right to withdraw from testing at any time, even without giving a reason. Furthermore, the participants will then have the opportunity to ask any questions and written informed consent form will be given by the participant once they are happy to participate in the study. Participants cannot take part unless a written informed consent form is completed and signed. This will be obtained through physical hand to hand. No children or adults at risk will participate in the study.

If participants are to be recruited from any of potentially vulnerable groups, give details of extra steps taken to assure their protection. Describe any arrangements to be made for obtaining consent from a legal representative.

***Copies of any written consent form, written information and all other explanatory material should accompany this application**. The information sheet should make explicit that participants can withdraw from the research at any time, if the research design permits. Remember to use meaningful file names and version control to make it easier to keep track of your documents.*

D.6 Describe whether participants will be able to withdraw from the study, and up to what point (e.g. if data is to be anonymised). If withdrawal is not possible, explain why not.

Any limits to withdrawal, e.g. once the results have been written up or published, should be made clear to participants in advance, preferably by specifying a date after which withdrawal would not be possible. Make sure that the information provided to participants (e.g. information sheets, consent forms) is consistent with the answer to D6.

The participants will be given a participant information form before signing the informed consent form to participate in the research project. It will be clearly stated on the information form how they will have the right to withdraw from the trial at any point without giving any reason to why they have come to this decision. Data collected thus far will be kept unless the participant specifically requests withdrawal of the data collected to date. If participants decide to withdraw after the data has been written up and published, it will not be possible for this to be deleted or destroyed.

D.7 How long will the participant have to decide whether to take part in the research?

It may be appropriate to recruit participants on the spot for low risk research; however consideration is usually necessary for riskier projects.

Once the participant has been contacted and asked to participate in the study, they will have access to forms and told the date of the trial will begin. They will have until that date to decide if they would like to participate, allowing them to have time to fully read and understand the research and their rights if they participate. Furthermore, they will have time to contact any research investigators for further information regarding the trial.

D.8 What arrangements have been made for participants who might have difficulties understanding verbal explanations or written information, or who have particular communication needs that should be taken into account to facilitate their involvement in

the research? *Different populations will have different information needs, different communication abilities and different levels of understanding of the research topic. Reasonable efforts should be made to include potential participants who could otherwise be prevented from participating due to disabilities or language barriers.*

As noted in D2 above, participants are required to understand and speak fluent English, to ensure full understanding and their own personal safety, throughout their participation in the programme. Demonstration of technique can be explained verbally and visually for certain aspects and participants have the opportunities to ask questions from the research team at any point. We are only recruiting trained participants which should negate difficulties in understanding what is expected from the programme.

D.9 Will individual or group interviews/ questionnaires discuss any topics or issues that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could take place during the study (e.g. during interviews or group discussions)? *The information sheet should explain under what circumstances action may be taken.*

	Yes		X	No

If yes, give details of procedures in place to deal with these issues.

D.10 Will individual research participants receive any payments, fees, reimbursement of expenses or any other incentives or benefits for taking part in this research?

	Yes		X	No

If Yes, please describe the amount, number and size of incentives and on what basis this was decided.

PART E: RESEARCH INVOLVING HUMAN TISSUES OR MATERIAL (leave blank if not applicable)

E.1 Will the research involve the use of any of the following? (Mark with X as appropriate)

	Foetal material
	The recently deceased
	Cadavers
X	Human bodily fluid
	Human tissue
	Human organs
	Human gametes

Go to Section F if the research does not involve any of the above material.

E.2 Will the material to be accessed be collected as part of this study or 3rd party accessed (E.g. material collected as part of another study or purchased)?

Venous blood will be collected as part of this trial by one of the test administrators who is trained in venepuncture technique.

If yes to 3rd party access, please provide details on appropriate consent for this use.

E.3 What type of tissue or material will be collected?

Venous blood

E.4 How will the tissue or material be collected and who will do this?

The primary supervisor (Dr James Bray) will collect venous blood samples and is trained in this technique.

E.5 How many samples will be collected?

There will be three draws in ~ 24 hrs, per participant, per trial. This frequency of draws is typical for a study of this design in the field of Sport and Exercise Nutrition.

E.6 How long will samples be stored?

Samples will be stored until the data has been analysed and written up and accepted for publication

E.7 Do you require a regulatory licence to use or store this material?

X	Yes		No

All material is expected to be stored in line with the Human Tissue Authority storage expectations.

E.8 Do you have the appropriate Health and Safety procedures in place for the researchers to handle the samples?

X	Yes		No

PART F: RESEARCH DATA

F.1 Explain what measures will be put in place to protect personal data. E.g. anonymisation procedures and coding of data. Any potential for re-identification should be made clear to participants in advance.

Personal information and data will be kept confidential at all times during and after the research. Participants will be allocated an anonymous participant code for the data and their personal details will be kept separately so that name or personal details are not associated with their data, to keep the data anonymous at all times. Informed consent and personal details will be kept in a locked cabinet only accessible to the research team and electronic data will be stored on a password protected computer or password protected university approved cloud storage platform.

Hard copies of participant's testing data will be collected, and data immediately transferred to digital copy and stored on a password protected computer and encrypted external hard drive. All information gathered in this study will be stored in line with the 2018 Data Protection Act and will be destroyed 5 years after the conclusion of this study. During this time the data may only be used by the research team for purposes related to the research question, but at no time will

personal information or data be revealed. The limits of confidentiality associated with this project will be made clear to participants prior to their participation (see Participation Information Sheet).

F2. What security measures are place to ensure secure storage of data at any stage of the research?

Provide details on where personal data will be stored, any of the following: (mark with X all that apply)

X	University approved cloud computing services
	Other cloud computing services
X	Manual files
	Private company computers
X	Portable devices
X	Home or other personal computers (not recommended; data should be stored on a University of Hull server such as your G,T, X or Z: drive where it is secure and backed up regularly).

Please complete the simplified DMP by following the link below and attach as a word document in the appendices. Please use the extended DMP if you are submitting an IRAS application.

https://libguides.hull.ac.uk/researchdata/plan

F.3 Who will have access to participant's personal data during the study?

Principle investigator and the rest of the research team detailed in sections A2, A3, and A4 above.

F.4 Where will the data generated by the research be analysed and by whom?

The data will be generated by Microsoft excel and SPSS or JASP by the student investigator/research team (as detailed in A2, A3, and A4 above).

F.5 Who will have access and act as long term custodian for the research data generated by the study?

Abbie Healy and the rest of the research team detailed in section A2, A3, and A4.

Data will be collected and secured at the University of Hull, by principal and co-investigators, and will only be accessible by members of the research team. Dr James Bray will act as long-term custodian for the research data generated in this study.

F.6 Have all researchers that have access to the personal data that will be collected as part of the research study, completed the University (or equivalent) data protection training?

X	Yes		No

It is mandatory that all researchers accessing personal data have completed data protection training prior to commencing the research.

F.7 Will the research involve any of the following activities at any stage (including identification of potential research participants)? (Select all that apply)

	Examination of personal records by those who would not normally have access
	Access to research data on individuals by people from outside the research team
X	Electronic surveys, please specify survey tool: JISC online survey tool
	Other electronic transfer of data
X	Use of personal addresses, postcodes, faxes, e-mails or telephone numbers
	Use of audio/ visual recording devices (NB this should usually be mentioned in the information for participants)

F.8 Are there any reasons to prevent or delay the publication of this research? E.g. Commercial embargoes, sensitive material.

	Yes		X	No

If yes, provide details:

F.9 Where will the results of this study be disseminated? (Select all that apply)

- [X] Conference presentation
- [X] Peer reviewed journals
- [X] Publication as an eThesis in the Institutional repository HYDRA
- [X] Publication on website
- [ ] Public data repository (e.g. the Open Science Framework OSF.org.io)
- [ ] Other publication or report, please state: _____
- [ ] Submission to regulatory authorities
- [ ] Other, please state: _____.
- [ ] No plans to report or disseminate the results

F.10 How long will research data from the study be stored?

5 years

F.11 When will the personal data collected during the study be destroyed and how?

Data will be destroyed 5 years after completion of the project, as detailed in the 2018 Data Protection Act/ the files will be deleted from the encrypted electronic media, and hard paper copies of the files will be disposed of appropriately as confidential waste, in accordance with University practices.

Researchers must comply with the General Data Protection Regulations that are live from May 2018.

PART G: CONFLICTS OF INTEREST

G.1 Will any of the researchers or their institutions receive any other benefits or incentives for taking part in this research over and above normal salary or the costs of undertaking the research?

	Yes		X	No

If yes, indicate how much and on what basis this has been decided

G.2 Is there scope for any other conflict of interest? *For example, could the research findings affect any ongoing relationship between any of the individuals or organisations involved and the researcher(s)? Will the research funder have control of publication of research findings?*

	Yes		X	No

If so, please describe this potential conflict of interest, and outline what measures will be taken to address any ethical issues that might arise from the research.

G.3 Does the research involve external funding? (Tick as appropriate)

X	Yes		No

If yes, what is the source of this funding?

Student Finance England

PART H: TRAINING

Please provide details of any training required to conduct this research by any member of the research team.

Training for JASP was conducted when the student investigator did their undergraduate degree.

PART I: DECLARATIONS

Declaration by Principal Investigator

1 The information in this form is accurate to the best of my knowledge and belief.
2. I take full responsibility for the information I have supplied in this document.
3. I undertake to abide by the University's ethical and health and safety guidelines, and the ethical principles underlying good practice guidelines appropriate to my discipline.
4. I will seek the relevant School Risk assessment/COSHH approval if required.
5. If the research is approved, I undertake to adhere to the project protocol, the terms of this application and any conditions set out by the Faculty Research Ethics Committee.
6. Before implementing substantial amendments to the protocol, I will submit an amendment request to the Faculty Research Ethics Committee seeking approval.
7. If requested, I will submit progress reports.
8. I am aware of my responsibility to be up to date and comply with the requirements of the law and relevant guidelines relating to security and confidentiality of participants or other personal data, including the need to register when necessary with the appropriate Data Protection Officer.
9. I understand that research records/data may be subject to inspection for audit purposes if required in future.
10. I take full responsibility for the actions of the research team and individuals supporting this study, thus all those involved will be given training relevant to their role in the study.
11. By signing the validation I agree that the Faculty Research Ethics Committee, on behalf of the University of Hull, will hold personal data in this application and this will be managed according to the principles established in the Data protection Act (1998).

Sharing information for training purposes: Optional – please mark with X as appropriate:

	I would be content for members of other Research Ethics Committees to have access to the information in the application in confidence for training purposes. All personal identifiers and references to researchers, funders and research units would be removed.

Principal Investigator

Signature of Principal Investigator: Abbie Healy
(This needs to be an actual signature rather than just typed. Electronic signatures are acceptable)

Print name: ABBIE HEALY Date: (dd/mm/yyyy): 07/02/2023

Supervisor of student research: I have read, edited and agree with the form above.

Supervisor's signature: Dr James Bray

(This needs to be an actual signature rather than just typed. Electronic signatures are acceptable)

Print name: Dr JAMES BRAY Date: (dd/mm/yyyy): 07/02/2023

B. FHS REC Risk Assessment Form

RISK IDENTIFICATION

Please identify all risks related to this research and indicate WHO is at risk and the measures that are in place or are required to mitigate these.

RISK(S)	**MEASURES IN PLACE / REQUIRED** ***(e.g. alternative work methods, training, supervision, protective equipment)***
Training / supervision: *(e.g. information or training required, level of experience, supervisor's input and oversight)*	Masters by Research student (principal investigator) will be given training for the administration of interventions and will be supervised by qualified staff from the University of Hull during the administration of the trial. Supervisors will provide input and assistance throughout the research process.
Location: *(e.g. remote area, laboratory, confined space, entry or exit, level of illumination, heating etc.)*	**Risk:** Slips, trips, falls, musculoskeletal injury. **Those at risk:** Study participants, members of the research team. **Control measures:** The trial will take place in the sports science laboratories the University of Hull, within the School of Sport, Exercise and Rehabilitation Sciences. Researchers will be familiar with safety procedures (such as fire evacuation) prior to commencing the study. All participants will be made aware of evacuation procedures prior to participation. Researchers will ensure that interventions will take place in rooms with adequate heating, lighting and space for participants and researchers. Moreover, a researcher qualified in first aid on site at all times. The researcher will demonstrate the correct and safe technique prior to the commencement of the exercise testing to reduce the risk of trips or falls during exercise. The testing / area to be used will be inspected for potential hazards prior to testing or exercising taking place. All potential hazards will be removed in advance by members of the research team or appropriate lab staff.

FHS RESEARCH ETHICS COMMITTEE

RISK ASSESSMENT FORM

Title of the research	Effectiveness of an omega-3 polyunsaturated fatty acid (n-3PUFA) supplement on endurance, performance and neuromuscular fatigue in cyclitis.
Name of Principal Investigator	Abbie Healy
Location of research	University of Hull

Brief description of research activity

This study aim is to investigate the effectiveness of a commercially available omega-3 and protein sports nutrition beverage on endurance performance, neuromuscular fatigue, and recovery in well-trained cyclists.

There will be a series of four laboratory visits; visit one, will consist of familiarisation, including, anthropometrical measures and a maximal oxygen uptake (VO2max) test, using a 50 watt (W), ramp protocol on a cycle ergometer. Visit two and three; will combine steady state cycling (75 mins at 60% VO_2max) with a 16.1km time trial (TT). Post warm-up, steady state cycle, and TT, 3 countermovement jumps (CMJ) will be performed to measure their neuromuscular fatigue. Between visit 2 and 3, participants will consume an omega-3 and protein beverage (1600 mg omega-3 + 20g protein) daily for 56-days/8weeks. Finally, the fourth visit will occur 24 hours after visit three, where the final blood sample will take place, as well as repeating the first laboratory visit to identify any observed changes in body composition and VO_2max. Pre-, post-, and 24 hours post TT, venous bloods will be taken (8ml). Some blood markers of interest include Interleukin-6 (IL-6) and interlekin-1 beta (IL-1b), creatine kinase (CK), cellular adhesion molecules and circulating microparticle population analysis.

Research processes: *(e.g. use of electrical systems, gas, liquids, tissue, potential for contamination, flammability etc.)*		**Risks:** data handling, supplementation protocol, testing protocol, slips, trips, feint/falls, musculoskeletal injury, cardiorespiratory complications. **Those at risk:** Participants, University, members of the research team. This study will require the use of a cycle ergometer coupled with a metabolic gas cart whilst conducting the VO_2max and performing trials, respectively. Furthermore, a mouthpiece, flow line will be connected to this metabolic gas cart - to allow for the determination of VO_2 and VO_2max. The use of a gauge needle to perform venepuncture during visits 2 and 3, will be used at three-time points; 1) pre-, 2) post-endurance trial and 3) 24hr post-TT. Minor gastro-intestinal discomfort such as slight bowel discomfort and/or feelings of nausea and bloating may be associated with the consumption of the omega-3 and protein beverage, however instances of this are highly unlikely. In rare cases, there is a possibility of allergy or overdose should the right protocol not be observed when using the supplement although instances of this are highly unlikely. Furthermore, researchers will not conduct evaluation of the effects of the omega-3 and protein beverage as it is a formulated product, however risks are expected to be rare. Also, participants will be asked to refrain from consuming any other omega-3 supplements, protein supplements, and fish oils which will reduce overdose likelihood. **<u>Control measures:</u>** Participants taking part in the study will all be familiarized with all physiological testing procedures and exercises prior to commencing the study. Individuals will then be watched completing the trial to ensure participants are safe. Participants will be monitored throughout testing and will be asked to stop if they're deemed unsafe to continue and can stop voluntarily at any point during the testing protocol.	

Loss or theft of an electronic storage device / computer and access to paper records.

All paper records will be stored in a locked filing cabinet in a secure location on campus, accessible only to the research team, and all electronic information will be stored on password protected electronic media (e.g. storage devices, computers). All information and data gathered during this research study will be stored in line with the 2018 Data protection Act and will be destroyed 5 years following the conclusion of the study. During that time the data may be used by members of the research team only for purposes appropriate to the research question, but at no point will personal information or data be revealed.

Personal information being revealed.

An anonymous participant code will be assigned to each participant to identify the data provided. Personal details will not be associated with the data and no information will be shared. Additionally participants will be made aware of the limits to confidentiality within this study prior to participation (see Participant Information Sheet).

Researcher may not be confident / competent in using the equipment used for testing and during the exercise programme.

All appropriate training will be provided to ensure the researcher is confident / competent in the use of testing and exercise equipment prior the commencement of data collection.

Explain Hazard: Falling over, tripping, sprains and strains caused by exercise. Strenuous exercise can potentially cause dizziness or fainting.

Control measure: Following the test participants will participate in a cool down. First aid is available on request from a technician in the lab. Have a first aider present on scene. Pre-medical questionnaires to screen for old or existing injuries, and a thorough warm-up and stretches prior to testing. The

	subject will continue to be closely monitored and supervised by one of the test administrators until physiological variables return to near normal. **Equipment may be faulty or damaged.** All equipment will be checked to ensure it is in safe working order prior to each testing session. All equipment to be sent home with participants will be checked prior to distribution. Participants will also be made aware of the need to check equipment prior to use in the home environment and between exercise sessions, to ensure it is in safe working condition.
Equipment use: *(e.g. manual handling, operation of emergency controls etc.)*	Cycle ergometer Mouthpiece Online gas-cart Heart rate monitor Gauge needle
Violence / upset / harm: *(e.g. potential for violence, sensitivity of topic, previous incidents etc.)*	Due to the nature of these exercise/performance trials, participants might feel nausea or overtired once the cycle has been completed. However, this is a sensation that participants are likelt to have experienced during their normal training and racing schedules. Researchers will make efforts to ensure participants are at ease and that they are aware of their right to withdraw from the trial at any time. Two members of the research team will be present at all times and a first aider will always be on hand. Security will also be contactable on the emergency number 5555 if on campus, or 999 if off campus.

CONTINUED.....	
Individuals: *(e.g. medical condition, young, inexperienced, disability etc.)*	Amateur cyclists, qualified professionals (e.g., Exercise, Physiologists), Masters by Research student. However, in the unlikely event of an incident a first aider will be present at all times. **Explain Hazard:** Not drinking enough to cope with the exercise. **Control measure:** Participants are informed to report in a hydrated state. Additionally water will be available throughout the visit and subjects will be advised to fully rehydrate following the testing procedure.
Work patterns: *(e.g. lone working, working out of hours, working off site, isolated or remote location etc.)*	All trials will be completed in areas with public access within working hours.
Other:	

Name of Principal Investigator:	Abbie Healy
Signature:	Abbie Healy
Date:	10/02/2023
Name of Supervisor (if relevant):	Dr James Bray
Signature:	Dr James Bray
Date:	10/02/2023

C. Data management Plan

University of Hull

Data Management Plan

Date	08/02/2023
Researcher(s)	Principal Investigator: Miss Abbie Healy Supervisors: Dr James Bray Dr Rebecca Vince
Project title	Effectiveness of omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation on endurance capacity, performance, and neuromuscular fatigue in cyclists.
Brief description	This study's aim is to investigate the effectiveness of a commercially available omega-3 and protein sport nutrition beverage on endurance performance, neuromuscular fatigue, and recovery in well-trained cyclists. There will be a series of four laboratory visits; visit one, will consist of familiarisation, including, anthropometrical measures and a maximal oxygen uptake (VO_2max) test, using a 50 watt (W), ramp protocol on a cycle ergometer. Visit two and three; will combine steady state cycling (75 mins at 60% VO_2max) with a 16.1km time trial (TT). Post warm-up, steady state cycle, and TT, 3 countermovement jumps (CMJ) will be performed to measure their neuromuscular fatigue. Between visit 2 and 3, participants will consume an omega-3 and protein beverage (1600 mg omega-3 + 20g protein) daily for 56-days/8weeks. Finally, the fourth visit will occur 24 hours after visit three, where the final blood sample will take place, as well as repeating the first laboratory visit to identify any observed changes in body composition and VO_2max. Pre-, post-, and 24 hours post TT, venous bloods will be taken (8ml). Some blood markers of interest include Interleukin-6 (IL-6) and interlekin-1 beta (IL-1b), creatine kinase (CK), cellular adhesion molecules and circulating microparticle population analysis.

Section 1: Project Information

A summary of the project details and associated data management requirements

Project title: Effectiveness of omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation on endurance capacity, performance, and neuromuscular fatigue in cyclists.
1.1 Project duration (aa/bb/cc-xx/yy/zz) 06/03/23-23/09/2023
1.2 Partners (if applicable) N/A
1.3 Brief description This study aim is to investigate the effectiveness of a commercially available omega-3 and protein sport nutrition beverage on endurance performance, neuromuscular fatigue, and recovery in well-trained cyclists. This is important for endurance athletes as they continually seek to enhance their exercise capacity and substrate utilisation, whilst accelerating their recovery from exercise (Hodgson, 1985). Omega-3 can reduce exercise-induced inflammation, improve skeletal muscle function, and energy availability in athletes (Simopoulos, 2007). Furthermore, prolonged omega-3 intake is suggested to alter ATP synthesis by increasing the capacity for mitochondrial reactive oxygen species emission without altering the content of oxidative products (Herbst et al., 2014; Baker, McCormick & Robergs, 2010). Suggesting that enhancing the oxidation of fatty acids, can improve an endurance athlete's ability to utilise substrates and maximise available stores of muscle glycogen. There will be a series of four laboratory visits; visit one, will consist of familiarisation, including, anthropometrical measures and a maximal oxygen uptake (VO2max) test, using a 50 watt (W), ramp protocol on a cycle ergometer. Visit two and three; will combine steady state cycling (75 mins at 60% VO_2max) with a 16.1km time trial (TT). Post warm-up, steady state cycle, and TT, 3 countermovement jumps (CMJ) will be performed to measure their neuromuscular fatigue. Between visit 2 and 3, participants will consume an omega-3 and protein beverage (1600 mg omega-3 + 20g protein) daily for 56-days/8weeks. Finally, the fourth visit will occur 24 hours after visit three, where the final blood sample will take place, as well as repeating the first laboratory visit to identify any observed changes in body composition and VO_2max.

Pre-, post-, and 24 hours post TT, venous bloods will be taken (8ml). Some blood markers of interest include Interleukin-6 (IL-6) and interlekin-1 beta (IL-1b), creatine kinase (CK), cellular adhesion molecules and circulating microparticle population analysis.

References:

Baker, J. S., McCormick, M. C., & Robergs, R. A. (2010). Interaction among skeletal muscle metabolic energy systems during intense exercise. *Journal of nutrition and metabolism*, 2010.

Herbst, E. A., Paglialunga, S., Gerling, C., Whitfield, J., Mukai, K., Chabowski, A., Heigenhauser, G. J., Spriet, L. L., & Holloway, G. P. (2014). Omega-3 supplementation alters mitochondrial membrane composition and respiration kinetics in human skeletal muscle. *The Journal of physiology*, 592(6), 1341–1352. https://doi.org/10.1113/jphysiol.2013.267336

Hodgson, D. R. (1985). Energy considerations during exercise. *Veterinary Clinics of North America: Equine Practice*, 1(3), 447-460.

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

1.4 Faculty or University requirements for data management

Completion of Faculty of Health Sciences Data Management Plan as part of this ethics application.

1.5 Funding body(ies)

Enhanced Omega-3- supplied the supplement beverage and possible biochemistries markers.

1.7 Budget (estimate if necessary)

Circa £500 for blood markers

1.8 Funding body requirements for data management

N/A. Applies specifically to funded projects.

Section 2: Data, Materials, Resource Collection Information

2.1 Brief description of data being created or compiled

Personal data

- Name, age, and contact information
- Consent forms
- Pre-exercise medical questionnaire

Research data

- Online survey data during the supplementation stage
  - Food diary
  - Wellness questionnaire
  - Illness questionnaire
  - Training log
  - Pre-exercise medical questionnaire
- Anthropometrical measures at laboratory visits 1 and 4
  - Heart rate
  - Weight
  - Height
  - Body composition
- Venous Blood sampling at laboratory visits 2 and 3 (pre-, post-, and 24 hours post TT)
  - Interleukin-6 (IL-6)
  - Interlekin-1 beta (IL-1b)
  - Creatine kinase (CK)
  - Clotting time
  - Erythrocyte membrane fatty acids profile
- Cycling data
  - VO_2 max
  - Power output
  - Speed
  - Time

2.2 Data collection process

Visit 1:

Participants will meet the researchers at the University of Hull. Comfortable sports clothing and sports shoes will be required. Participants will be encouraged to attend the session in a well-hydrated state, they will be asked to refrain from any strenuous activity for 48 hours before the visit. Regular hand sanitisation will be completed throughout the session and in the various locations in which the testing will take place. The researcher will brief participants on how the session is going to run and answer any questions or concerns that they might have. If they still meet the inclusion criteria for the study following completion of the pre-exercise medical questionnaire, and confirming they have no known allergies or intolerances of the supplement and are currently not taking any other protein supplements or fish oils, then their resting heart rate, weight, height, and body composition will be recorded. Subsequently, participants will be asked to complete an incremental cycling test to exhaustion (VO2max). This will involve participants undertaking a 10-minute warm up with a preferred workload <

150Watts (W) on the cycle ergometer. After the warm up period, the VO2max protocol commenced, and was increased 50W every minute until volitional exhaustion. Peak oxygen consumption, peak workload, submaximal and peak heart rate and participant rating of perceived exertion (RPE) were assessed. During this test, the aforementioned data were collected automatically by the metabolic gas-cart. Participants were allowed to cycle at any pace 70-120 cadence rpm. These measures (skinfolds and VO2max) were repeated after the 56-day supplementary period (visit 4).

Visit 2:

Workload for the submaximal exercise component was set at 60% VO_2max derived from the maximal oxygen consumption test. This same workload was used after the supplementation trial, allowing for direct comparisons to be made for pre- to post-supplementation of O_2 consumption, HR and RPE, respectively. After the 75-minute submaximal exercise period, participants then completed the 16.1km time trial (TT). All participants will be asked to report their RPE after every 2km according to the Borg scale. Furthermore, during the trial their heart rate will be monitored and recorded every 2km. Time, speed, and power output will be recorded at the end of the session by the researchers using the specific ergometer software. Venous bloods will be taken from the participants pre-, post-, and 24 hours post TT. Post warm-up, post the steady state cycle, and post TT, the participants will be asked to complete a countermovement jump (CMJ) protocol which will measure their neuromuscular fatigue. This will involve participants to perform 3 vertical jumps by quickly squatting 90 degrees and then jumping as high as possible, with the peak value being recorded. Pre-exercise and 24-hours post exercise, participants will be asked to complete a wellbeing questionnaire to track any changes in mood.

Visit 3:

All procedures will be identical to visit 2, however this will be after a 56 days/8 weeks omega-3 and protein supplementation.

Visit 4:

This visit will occur 24 hours post the completion of the participants 3rd visit to the laboratory, where a trained technician will complete the final blood sample. Then as detailed previously, measures of skinfolds and re-test of VO_2max will be repeated allowing for direct comparisons to be made for pre- to post-supplementation of sum of skinfolds, peak power, O_2 consumption, HR and RPE, respectively.

Online survey data will be collected via JISC.

2.3 Are there existing forms of the data that will be used within this research project, or which will be used as the basis for the research? If so, provide a brief description and citation.

The study design has been chosen to build on previous research investigating the effectiveness of omega-3 on endurance performance, neuromuscular fatigue, or recovery in athletes.

- Drobnic, F., Rueda, F., Pons, V., Banquells, M., Cordobilla, B., & Domingo, J. C. (2017). Erythrocyte omega-3 fatty acid content in elite athletes in response to omega-3 supplementation: a dose-response pilot study. *Journal of Lipids*, *2017*.
- Hansen, M. W., Ørn, S., Erevik, C. B., Bjørkavoll-Bergseth, M. F., Skadberg, Ø., Melberg, T. H., ... & Kleiven, Ø. (2021). Regular consumption of cod liver oil is associated with reduced basal and exercise-induced C-reactive protein levels; a prospective observational trial: A NEEDED (The North Sea Race Endurance Exercise Study) 2014 sub-study. *Journal of the International Society of Sports Nutrition*, *18*(1), 51.
- Kyriakidou, Y., Wood, C., Ferrier, C., Dolci, A., & Elliott, B. (2021). The effect of Omega-3 polyunsaturated fatty acid supplementation on exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition*, *18*(1), 9.
- Thielecke, F., & Blannin, A. (2020). Omega-3 fatty acids for sport performance—are they equally beneficial for athletes and amateurs? a narrative review. *Nutrients*, *12*(12), 3712.
- 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.
- 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.

2.4 Will data be available in electronic format (if so then state format(s))?

Yes, pre-exercise medical questionnaires and informed consent will be completed by participants as a physical paper document. These will be stored in a locked cabinet at the University of Hull, which only the researcher can access.

Online survey data will be held for the duration of the survey on JISC. The data will then be transferred in MS excel format to the encrypted laptop and then input into SPSS statistical software for analysis.

2.5 Will the data be available in non-digital form (if so then state format(s))?

All data will be comprehensible to the extent that the researcher involved in the study will have access the data. Data will not be accessible to outside parties.

2.6 Will the data stand alone and be comprehensible to a third party or be accompanied by explanatory documentation (e.g., a data dictionary)?

Research data will be recorded using an encrypted laptop and be transcribed to be analysed. Electronic files of data will contain pseudonyms or codes and be stored in separate files on the researcher's encrypted laptop. The completed data will be held in

electronic format on secure drives at the University of Hull in a format that will clearly denote the nature of the research and the project to which they relate.

2.7 Describe the quality assurance process for data management

The progress of the project will be monitored during monthly supervision sessions. The quality of the process for the data management will be overseen and monitored by the researcher.

Section 3: Ethics, Intellectual Property

3.1 How will the ethical aspects of data storage and subsequent access be addressed?

To ensure that no individual participant can be identified from their data, during the data collection phase all participants will be identified via a personal four-digit alphanumeric code. A password-protected file containing the participants contact details and personal code will be safely secured and only accessible to the researcher. This file will only be used to ensure the participant's data can be matched to them at each visit. Once the study has been concluded, and quality assurance processes have been completed, this file will be deleted, meaning that all remaining data is anonymized.

Paper based data will be stored in the study master file in a locked room on the University campus and subsequently backed up electronically to prevent data loss. All electronic data will be stored on University password protected computers and backed up on the university approved cloud storage platform (Box.com). All primary study data will be destroyed after 5-years.

All participants will be provided with the participant information sheet, which outlines how their data will be used, and what their choices are regarding their personal data. Before any data is collected, participants will be given the opportunity to ask any relevant questions, before signing an informed consent form to show they agree to these processes.

The on-line survey data will not contain any identifiable information.

3.2 Will the data comply with relevant legislation such as Data Protection Act, Copyright, Design and Patents Act, Freedom of Information Act, etc.? Yes, as highlighted to participants in the Participant Information Sheet, all data will be processed in accordance with the UK-GDPR and the data Protection Act 2018.
3.3 If several partners are involved how will compliance with 3.1 and 3.2 be assured? N/A

Section 4: Access and Use of Information

4.1 Are you required, or do you intend, to share the data, and with whom? If so, when? It is intended that the data collected will be used by the researcher for the requirements for a Masters by Thesis degree, in scientific research articles, and with Enhanced Omega-3 (provider of the supplement beverage). It is also envisaged that the data will be disseminated in the form of a peer-reviewed academic journal once data collection/analysis is complete.
4.2 If 'yes' to 4.1, in what format will data be shared? The research findings will be submitted to a relevant journal for publication. It is not intended that raw data sets (i.e., data from cycle computer head-unit) will be shared.
4.3 Will the data have to be stored and/or made accessible for a specific period (if so, how long)? The anonymised research data will be stored for 5 years beyond the end of the research, in line with the University of Hull regulations.
4.4 Who may need or wish to have access to the data? The researcher will need access to the data. Peer reviewed academic journals may require raw data be made available as part of the publication process. However, all data will remain anonymised with a unique study code know only to the researcher.
4.5 How do you anticipate the data being used subsequent to the project?

The data will be used in a number of ways after the project. Primarily this will be used to satisfy the requirements for a Masters by Thesis degree and in scientific research articles. The data may also be used at a conference presentation, and to help inform future research design.

Section 5: Storage and Backup of Data

5.1 Where and how will the data be stored **during the lifespan of the project**?

Hardcopy data (consent forms with identity code, and pre-exercise medical questionnaires) will be stored in the room of the principal investigator at the University of Hull, in a locked cabinet.

Electronic files (physiological data) will be password protected and stored on a university approved cloud-based online storage platform (e.g. Box.com).

5.2 Where and how will the data be stored **on completion of the project**?

Hardcopy data (consent forms with identity code, and pre-exercise medical questionnaires) will be stored in the room of the principal investigator at the University of Hull, in a locked cabinet.
Electronic files (physiological data) will be password protected and stored on a university approved cloud-based online storage platform (e.g. Box.com).

5.3 What provision is being made for backup of the data?

All data will be backed up electronically on password protected University computers. All data will be saved by uniquely numbering each version with the data (dd/mm/yy), so that data can easily be recovered if needed. Each version will be accessible using a university approved cloud-based online storage platform (e.g., Box.com), which allows for access to previous versions.

5.4 Will different versions of the data be stored? If so, what frequency of versioning will be appropriate?

As detailed above.

Section 6: Archiving and Future Proofing of Information

6.1 What is the long-term strategy for future proofing of the data?

Long term storage will be on secure University drives under the custody of the research supervisor.

If the research is submitted to a journal, then it may be available in an electronic copy.

6.2 How will the data be managed after the life of the project, for how long and in what format (NB this section refers to the detail of preservation and archiving actions, not just how it will be stored – this is addressed in section 5.2)?

Data will be kept for 5 years after the completion of the project in the following format:

Hardcopy data (consent forms with identity code, and pre-exercise medical questionnaires) will be stored in the room of the principal investigator at the University of Hull, in a locked cabinet.

Electronic files (physiological data) will be password protected and stored on a university approved cloud-based online storage platform (e.g. Box.com).

6.3 If the data include confidential or sensitive information, how will these data be managed to prevent possible future breaches?

All data collected will be confidential and pseudonyms or codes will be allocated to participants, and this will be used on each piece of data. Electronic data will be password protected and stored on the researcher's encrypted PC as well as on the university approved cloud-based online storage platform (e.g., Box.com).

6.4 If metadata or explanatory information is to be archived, how will this be linked to the data?

N/A

6.5 How will the data be cited?

N/A

Section 7: Resourcing of Data Management

7.1 List the specific staff who will have access to the data and denote who will have the responsibility for data management.

Dr James Bray will be running the day-to-day operations of the research and will be responsible for the immediate data management.

7.2 How will the data management described in this document be funded?

Departmentally, consistent with current practice for research staff. However, no additional costs for data management are anticipated.
7.3 How will data storage be funded? No additional costs of storage are anticipated, and data will be held on University secure drivers (and Box.com) for the prescribed storage period.

Section 8: Review of Data Management process

8.1 How will the data management plan be adhered to? Throughout the research, the data management plan will be used by the researcher to guide the data collection and management process.
8.2 Who will review the data management plan? What is the schedule for this review? As the principal investigator, Dr James Bray will be lead for reviewing the data management plan. This will take place periodically during the research process, to ensure the data management plan is being adhered to. There will also be monthly research meetings to keep the student researcher on-track. The researcher will seek advice from the FHS Ethics committee if any important matters arise.

Section 9: Statements and Personnel Details

9.1 Statement of agreement

I/we agree to the specific elements of the plan as outlined:

Principal investigator or PhD supervisor

Title	Miss
Designation	Principal investigator
Name	Abbie Healy
Date	10/02/2023
Signature	Abbie Healy

Researcher

Title	Dr
Designation	Primary researcher
Name	James Bray
Date	10/02/2023
Signature	James Bray

Researcher

Title	Dr
Designation	MSc Supervisor
Name	Rebecca Vince
Date	14/02/23
Signature	Rebecca Vince

Researcher

Title	Dr
Designation	Researcher
Name	Leigh Madden
Date	14th February 2023
Signature	Leigh Madden

D. FHS REC- Form C

FHS RESEARCH ETHICS COMMITTEE FORM C: Notice of Substantial Amendment

Principal Investigator contact details	
Name:	Miss Abbie Healy
Address:	Sport, Exercise and Rehabilitation Sciences University of Hull
Telephone	
Email:	a.healy-2018@hull.ac.uk

Full title of study	Effectiveness of omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation on endurance capacity, performance, and neuromuscular fatigue in cyclists.
REC reference number (from original authorisation letter)	HS 22-23.50
Date study commenced	Data collection has not commenced yet
Insert amendment number & date	Amendment number 1 - 17/05/23

Tick all that apply	Type of amendment	Effective date for amendment
x	Changes to the design or methodology of the study, or to background information affecting its scientific value	22/05/23
☐	Changes to the procedures undertaken by participants; any change relating to the safety or physical or mental integrity of participants, or to the risk/benefit assessment for the study	
☐	Significant changes to study documentation such as participant information sheets, consent forms, questionnaires, letters of invitation, letters to GPs or other clinicians, information sheets for relatives or carers etc.	
☐	Appointment of a new chief investigator	
☐	Temporary halt of a study to protect participants from harm, and the planned restart of a study following a temporary halt;	
☐	A change to the definition of the end of the study	
☐	Any other significant change to the protocol or the terms of the original approved ethics application	

Is this a modified version of an amendment previously notified to the REC and given an unfavourable opinion?	○ Yes ◉ No

DECLARATIONS:

I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.

I consider that it would be reasonable for the proposed amendment to be implemented.

Principal Investigator:

Abbie Healy

Signature of Principal Investigator: .....

(This needs to be an actual signature rather than just typed. Electronic signatures are acceptable)

Print name: Abbie Healy Date: (dd/mm/yyyy) 18/05/2023

Supervisor of student research: I have read, edited and agree with the form above.

Supervisor's signature: James Bray .....

(This needs to be an actual signature rather than just typed. Electronic signatures are acceptable)

Print name: J.Bray Date: (dd/mm/yyyy) 18/05/2023

This application should be emailed to the ethics submission email address
FHS-ethicssubmissions@hull.ac.uk

No actions relating to the amendment should be Undertaken until approval has been obtained.

List of attached documents (these should be added to the end of this document)

Document	Version	Date
Healy_FHS REC Form A1 V.2	V2.0	17/05/23
UoH PIS Form- Information sheet for participants	V2.0	17/05/23

E. Recruitment Poster and participant Letter of Invitation

Recruitment Poster 2023- Version 1.0

We are looking for participants to participate in a minimum of a 3-month trial (including a 52-days/8-week supplementation phase) which has been designed to investigate the effectiveness of an omega-3 and protein sports nutrition beverage on the endurance capacity, muscle fatigue, and recovery in cyclists.

We need you if you:

- Aged >18 years
- Participate in cycling
- Train at an amateur level (4-6 hours a week) OR semi-professionally level (7+ hours a week)
- Train at least >4 hours per week
- Accustomed to high intensity exercise
- Free from any existing medical conditions/injuries at time of testing
- Capable of giving informed consent
- Able to understand and communicate effectively in English
- Apparently healthy

If you are interested and would like to know more and may be willing to participate, please contact:

Dr James Bray/ J.Bray@hull.ac.uk/

Participant Letter of Invitation Version 1.0 – 08/02/2023

Project Title	Effectiveness of omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation on endurance capacity, performance, and neuromuscular fatigue in cyclists
Supervisor	Name: Dr James Bray Email address: J.Bray@hull.ac.uk
Co-Supervisor	Name: Dr Rebecca Vince Email address: rebecca.vince@hull.ac.uk
Student Investigator	Name: Abbie Healy Email address: a.healy-2018@hull.ac.uk

Dear Sir or Madam

This is a letter of invitation to enquire if you would like to take part in a research trial investigating the effectiveness of omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation on the endurance capacity, performance, and neuromuscular fatigue in well-trained cyclists supervised by the University of Hull.

Before you decide if you would like to take part, it is important for you to understand, why the project is being done and what it will involve. Please take time to carefully read the Participant Information Sheet on the following pages and discuss it with others if you wish. Ask me if there is anything that is not clear, or if you would like more information.

If you would like to take part, please complete the Informed Consent Declaration form.

Please do not hesitate to contact me if you have any questions.

Yours faithfully,

Abbie Healy

INFORMATION SHEET FOR PARTICIPANTS

UNIVERSITY OF HULL

Title of study

Effectiveness of omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation on endurance capacity, performance, and neuromuscular fatigue in cyclists

I would like to invite you to participate in a research project which forms part of my requirements for a Masters by Thesis degree in scientific research. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask me if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

Currently there is limited research about the effectiveness of omega-3 supplementation in athletes, with the majority on healthy individuals. Therefore, this research is addressing the gap in the literature by this research design. The purpose of this research is to investigate the effectiveness of an omega-3 and protein sports nutrition beverage on the endurance capacity, muscle fatigue, and recovery in well-trained cyclists. We are hoping that the data collected from this study may help inform nutritional practices surrounding how endurance athletes (and support staff), may wish to utilise supplementary omega-3 to enhance their endurance capacity, reduce muscle fatigue and markers of stress and accelerate recovery.

Why have I been invited to take part?

You are being invited to participate in this study because you have been identified as being eligible to participate. This study is recruiting endurance cyclists, who are ether at an amateur or semi-professional level.

Please note you should not participate if you meet any of the following exclusion criteria:

- You must not be taking any supplements/ergogenic aids (i.e., omega-3) within the last 6-months
- You must not have a known allergy/intolerance to omega-3 or any other ingredient in the product or have experienced any adverse event(s), to its supplementation
- You must train >4 hours per week and be accustomed to high intensity exercise
- You must not be under the age of 18 years old and be able to provide informed consent for this trial
- You must be free from any existing medical conditions/injuries at the time of testing and be apparently healthy
- You are already participating in a separate research study

What will happen if I take part?

If you choose to take part in the study you will be asked to attend the laboratory on four separate occasions. Visit one will consist of a familiarisation session, anthropometrical measures, and maximal oxygen uptake (VO_2max) test. Visit two will combine steady state cycling (60% VO_2max) with a 16.1 km time trial (TT), with the assessment of neuromuscular fatigue (via a countermovement jump protocol). Visit two and three will be interspersed by 56 day/8-week omega-3 and protein beverage supplementation period which will be taken daily. During this supplementation period, you will be asked to complete a food diary, training log, and visual analogue scale. Upon completion of the supplementation period, you will return to the laboratory for visit three where it will be a repeat of visit two. 24 hours later, you will return to the lab for a repeat of visit 1.

Laboratory visit(s)

Visit 1 and 4:

The pretesting will consist of resting heart rate, weight, height, and body composition. Body composition will measured using ISAK 8-site method by an accredited technician. Subsequently, you will be asked to complete an incremental cycling test to exhaustion (VO2max). This will involve you undertaking a 10-minute warm up with a preferred workload < 150Watts (W) on the cycle ergometer. After the warm-up period, the VO2max protocol will be commenced, and will be increased 50W every minute until volitional exhaustion. Peak oxygen consumption, peak workload, submaximal and peak heart rate and participant rating of perceived exertion (RPE) will be assessed. During this test, the aforementioned data will be collected automatically by the metabolic gas-cart. You will be allowed to cycle at any pace 70-120 cadence rpm.

Visit 2 and 3:

Workload for the submaximal exercise component will be set at 60% VO_2max derived from the maximal oxygen consumption test. After the 75-minute submaximal exercise period, you then completed the 16.1 km time trial (TT). At three time-points during these visits you will also be required to perform three maximal countermovement jumps at three time points (post warm-up, post submaximal exercise and post TT) for the indirect assessment of neuromuscular fatigue. You will be asked to report your RPE after every 2 km according to the Borg scale. Furthermore, during the trial your heart rate will be monitored and recorded every 2 km. Time, speed, and power output will be recorded at the end of the session by the researchers using the specific ergometer software. Venous bloods will be taken pre-, post-, and 24 hours post TT. During the trial period you will be

asked to complete a wellness (including URTI symptoms and muscle soreness) questionnaire pre-exercise and 24-hours post-exercise.

Omega-3 and protein supplementation period:

Furthermore, you will be asked to refrain from consuming >3 servings of fish per week, including any omega-3 supplements not supplied by the trial's supervisors. This will be monitored by you keeping a food diary. You will be asked to track your overall daily food intake twice a week, once on the weekday and once on the weekend, for eight weeks. For 56 days/8 weeks, you will consume a commercially available omega-3 and protein beverage (1600 mg omega-3 + 20 g protein) followed by completing a visual analogue scale, daily. Furthermore, you will be asked to complete a training log to allow for weekly training load to be calculated (duration *HR).

Blood Sampling:

Venous bloods will be taken a total of three times per experimental trial at; baseline, after exercise, and 24-hours post exercise. Venous blood samples will be assessed for markers of cellular stress, injury and recovery, to look to see the effects of exercise and the supplement on markers of inflammation, muscle damage, oxidative stress, cardiovascular markers and tissue repair. A resting 20 ml blood sample from the antecubital vein will be collected at each blood draw during visits 2 and 3 (120 ml over the whole trial). Samples will be collected and stored at –80 degrees for later analysis of the markers above directly and in lab-based models once all trials have been completed.

Do I have to take part?

Participation is completely voluntary. You should only take part if you want to and choosing not to take part will not disadvantage you in any way. Once you have read the information sheet, please contact us if you have any questions that will help you make a decision about taking part. If you decide to take part we will ask you to sign a consent form and you will be given a copy of this consent form to keep.

What are the possible risks of taking part?

Falling over, tripping, sprains and strains caused by exercise. Strenuous exercise can potentially cause dizziness or fainting: Following the test, participants will participate in a cool down. A first aider will be present during the testing. Pre-medical questionnaires to screen for old or existing injuries, and a thorough warm-up and stretches prior to testing will be performed to minimise risk and participants are used to this type of activity. The subject will continue to be closely monitored and supervised by one of the test administrators until physiological variables return to near normal.

Venous blood sampling may leave bruises around the puncture site: Procedure only to be performed by those who have attended an appropriate course and have undergone the required supervised practice. Pressure applied to sample site after needle removal.

Strenuous prolonged exercise has the potential to cause cardiorespiratory problems: Pre-exercise screening, including age, family health history and current level of physical activity to identify 'at risk' individuals. Subjects should be accustomed to regular high intensity exercise. The test will be terminated at the subject's request or upon reaching 85% of maximal heart rate. Subject monitored at all times and first aider should be available.

Puncturing of skin during venous blood sampling cause risk of infection to subject and/or cross contamination between subject and administrator: Subjects complete a pre-screening questionnaire to highlight any contraindications to the procedure in the first instance. All subjects and experimenters to wash hands thoroughly before testing. SOP for blood sampling to be strictly adhered to. This includes thorough hand washing, use of rubber gloves, safety specs, lab coats, proper post sampling cleaning procedures and clinical and sharps waste disposal procedures. Samples processed according to well established procedures and manufacturers instructions. In case of sharps injury follow procedures provided in the lab manual and displayed in the lab. If in doubt contact occupational health department or failing that seek medical advice immediately

Spillage of participants blood whilst taking the venous blood samples: Sampling should be taken over an easily cleanable surface. Protective clothing (lab coat, gloves, safety specs) must be worn at all times when dealing with spillages. Spillages must be cleaned up thoroughly and as soon as possible following lab procedures. Clinical waste bin nearby. Follow guidelines issued by department regarding COSHH.

Any nausea or vomiting or fainting/fall caused by having a blood sample taken: Participants are advised not to look as the sample is being taken and must be seated and watched closely in case they feel faint. Clinical waste bag and cleaning fluids (Milton / Virkon Soln) available to deal with any spillage according to relevant departmental COSHH form.

Allergy/overdose: The test administrator must explain the supplementation protocol clearly. The subjects must keep a food diary to ensure they are not consuming a diet already high in omega-3. The subject must confirm to the test administrator that he does not have an allergy/intolerance to the supplement prior to testing. If allergy/overdose is suspected seek emergency medical attention immediately.

What are the possible benefits of taking part?

As a result of participating in this study you will receive a personalised report detailing your maximal oxygen uptake (VO_2max), which can be used to help inform your own training. You will also receive 8-weeks of omega-3 and protein supplementation. Upon completion of this study and the data analysis, you will be able to see if this supplement improved your endurance performance, which in turn could inform your nutrition race practices.

How will we use information about you?

Your results will be allocated with an anonymous code and will therefore keep your results confidential. Written consent forms and personal details will be kept away from results and locked in a filing cabinet. Your personal data will be deleted upon study completion. Results will be processed and kept on a password protected memory stick/ laptop. Your data will be processed in accordance with the UK-GDPR and the Data Protection Act 2018. The anonymised research data will be archived for 5-years from the completion of the study. Electronic data will be saved in a secure location in accordance with University regulations for storage and research data. At the end of the retention period, all data will be destroyed in line with University regulations. Only the student investigator and the research team will come into contact and use the results. Also no personal details and results will be revealed throughout the study to anyone other than the participant and investigators. If the results from this study are published or presented in any form, the participants will be unidentifiable through coding of results.

What are your choices about how your information is used?

You are free withdraw at any point of the study, without having to give a reason but we will keep information about you that we already have.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

Where can you find out more about how your information is used?

You can find out more about how we use your information:

- By asking one of the research team
- By contacting the University of Hull Data Protection Officer by emailing dataprotection@hull.ac.uk or by calling 01482 466594 or by writing to the Data Protection Officer at University of Hull, Cottingham Road, Hull, HU6 7RX
- By reviewing the University of Hull Research Participant privacy notice: https://www.hull.ac.uk/choose-hull/university-and-region/key-documents/docs/quality/research-participant-privacy-notice.pdf

Data Protection Statement

The data controller for this project will be the University of Hull. The University will process your personal data for the purpose of the research outlined above. The legal basis for processing your personal data for research purposes under GDPR is a 'task in the public interest'

If you are not happy with the sponsor's response or believe the sponsor processing your data in a way that is not right or lawful, you can complain to the Information Commissioner's Office (ICO) (www.ico.org.uk or 0303 123 1113).

What will happen to the results of the study?

The results of the study will be summarised as part of the requirements for my Masters by Research Thesis and may be published in scientific journals or presented at conferences. Research data will be available in a summary report to participants following participation in the study, upon request. Any published manuscripts and reports will also be available to participants, upon request. If the thesis is published or presented in any form, the participants will be unidentifiable through coding of results.

Who has reviewed this study?

Research studies are reviewed by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and been given a favourable opinion by **the Faculty of Health Sciences Ethics Committee, University of Hull.**

Who should I contact for further information?

If you have any questions or require more information about this study, please contact me using the following contact details:

Email: J.Bray@hull.ac.uk

What if I have further questions, or if something goes wrong?

If you wish to make a complaint about the conduct of the study, you can contact the University of Hull using the details below for further advice and information:

In the first instance please contact Dr James Bray (J.Bray@hull.ac.uk).

Alternatively please contact a.healy-2018@hull.ac.uk

Thank you for reading this information sheet and for considering taking part in this research.

G. Informed Consent Form GDPR FHS

Version number and date: V1, 08/02/2023

UNIVERSITY OF HULL

CONSENT FORM

Title of study: Effectiveness of omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation on endurance capacity, performance, and neuromuscular fatigue

Name of Researcher: Miss Abbie Healy

Please initial box

1. I confirm that I have read the information sheet dated 09/02/2023 version 2 for the above study. I have had the opportunity to consider the information, ask questions and have had any questions answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. I understand that the data I have provided up to the point of withdrawal will be retained. ☐

3. I understand that the research data, which will be anonymised (not linked to me), will be retained by the researchers and may be shared with others and publicly disseminated to support other research in the future. ☐

4. I understand that my personal data will be kept securely in accordance with data protection guidelines, and will only be available to the immediate research team ☐

5. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

H. Pre-Exercice Medical Questionnaire

Pre-Exercice Medical Questionnaire V.1 – 08/02/2023

The information in this document will be treated as strictly confidential

Name:

Date of Birth: Age: Gender:

Blood pressure: Resting blood pressure:

Height (cm): Weight (Kg):

Please answer the following questions by putting a circle around the appropriate response or filling in the blank.

1. How would you describe your present level of **exercise** activity?

   Sedentary / Moderately- active / Active / Highly active

2. Please outline a typical week's exercise activity

3. How would describe your present level of **lifestyle** activity?

   Sedentary / Moderately- active / Active / Highly active

4. What is your occupation?
5. How would you describe your present level of fitness?

   Unfit / Moderately fit / Trained / Highly trained

6. Smoking Habits

Are you currently a smoker? Yes / No

How many do you smoke per day

Are you a previous smoker? Yes / No

How long is it since you stopped? years

How many did you smoke? per day

7. Do you drink alcohol? Yes / No

If you answered **Yes** and you are male, do you drink more than 28 units a week?

Yes / No

If you answered **Yes** and you are female, do you drink more than 21 units a week?

Yes / No

8. Have you had to consult your doctor within the last six months? Yes / No

If you answered **Yes**, have you been advised **not** to exercise? Yes / No

9. Are you presently taking any form of medication? Yes / No

If you answered **Yes**, have you been advised **not** to exercise? Yes / No

10. Do you have a history of fainting during or following exercise? Yes / No

If **Yes**, please provide details:

11. Do you have any known allergies? Yes / No

If **Yes**, please provide details:

12. Please outline any supplementation you are currently taking, and the reason for use

	Estimation of use					Reason for use					
Supplement	Never	Once a month	Once a week	Few times a week	Daily	Performance enhancer	General health	Greater energy	Increased endurance	Greater muscle mass	Other
Vitamin A											
Vitamin C											
Vitamin D											
Vitamin E											
Multivitamin											
Calcium											
Chromium											
Creatine											
Folatc (Folic acid)											
Iron											
Magnesium											
Potassium											
Phosphate											
Zinc											
Other											

13. To the best of your knowledge do you, or have you ever, or have a family history:

A Diabetes? Yes / No

B Asthma? Yes / No

C Epilepsy? Yes / No

D Bronchitis? Yes / No

E *Any form of heart complaint? Yes / No

F Raynaud's Disease Yes / No

G *Marfan's Syndrome? Yes / No

H *Aneurysm / embolism? Yes / No

I Anaemia Yes / No

14. *Are you over 45, and with a history of heart disease in your family?

Yes / No

15. Do you currently have any form of muscle or joint injury? Yes / No

If you answered **Yes**, please give details

16. Have you had to suspend your normal training in the last two weeks?

Yes / No

If the answer is **Yes** please give details

17. * Please read the following questions:

A. Are you suffering from any known serious infection? Yes / No

B. Have you had jaundice within the previous year? Yes / No

C. Have you ever had any form of hepatitis? Yes / No

D. Are you HIV antibody positive Yes / No

E. Have you had unprotected sexual intercourse with any person from an HIV high-risk population? Yes / No

F. Have you ever been involved in intravenous drug use? Yes / No

G. Are you haemophiliac? Yes / No

18. As far as you are aware, is there anything that might prevent you from successfully completing the tests that have been outlined to you? Yes / No

IF THE ANSWER TO ANY OF THE ABOVE IS YES:

a) Discuss with the test administrators or another appropriate member of the department.

b) Questions indicated by (*) answered yes: Please obtain written approval from your doctor before taking part in the test.

PLEASE SIGN AND DATE AS INDICATED

Participant Signature: Date:

Supervising staff member: Date:

Parent (if minor): Date:

THIS SECTION IS ONLY REQUIRED FOR RETURNED VISITS!

For any future testing sessions, it is necessary to verify that the responses provided above are still valid, or to detail any new information. This is to ensure that you have had no new illness or injury that could unduly increase any risks from participation in the proposed physical exercise.

ANSWER THE FOLLOWING QUESTIONS AT EACH REPEAT VISIT.

Is the information you provided above still correct, and can you confirm that you have NOT experienced any new injury or illness which could influence your participation in this exercise session?

Repeat 1	Yes / No	Signature:	Date:
*Additional information required:			
Repeat 2	Yes / No	Signature:	Date:
*Additional information required:			
Repeat 3	Yes / No	Signature:	Date:
*Additional information required:			

I. Food Diary Template

Food Diary Version 1.0 – 08/02/2023

It is important to have a low omega-3 diet during this trial. We are asking you to keep track of your daily food intake twice a week, once on the weekday and once on the weekend, for eight weeks. Please provide as much detail as possible. To calculate macronutrients, MyFitness pal is a recommended app which calculates this all for you.

	Breakfast	**Lunch**	**Dinner**	**Snacks**	**Liquids**
Week One Date:					
Day 1 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 2 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 3 (weekend)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
	Breakfast	**Lunch**	**Dinner**	**Snacks**	**Liquids**
Week Two Date:					
Day 1 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:

Day 2 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 3 (weekend)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
	Breakfast	**Lunch**	**Dinner**	**Snacks**	**Liquids**
Week Three Date:					
Day 1 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 2 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 3 (weekend)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
	Breakfast	**Lunch**	**Dinner**	**Snacks**	**Liquids**
Week Four Date:					
Day 1 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:

Day 2 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 3 (weekend)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
	Breakfast	**Lunch**	**Dinner**	**Snacks**	**Liquids**
Week Five Date:					
Day 1 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 2 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 3 (weekend)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
	Breakfast	**Lunch**	**Dinner**	**Snacks**	**Liquids**
Week Six Date:					
Day 1 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:

Day 2 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 3 (weekend)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
	Breakfast	**Lunch**	**Dinner**	**Snacks**	**Liquids**
Week Seven Date:					
Day 1 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 2 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 3 (weekend)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
	Breakfast	**Lunch**	**Dinner**	**Snacks**	**Liquids**
Week Eight Date:					
Day 1 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:

Day 2 (weekday)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:
Day 3 (weekend)	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Time: Protein Intake (g): Carb Intake (g): Fat Intake (g): Omega-3 Intake (g): Description:	Times: Portion size (in ml): Description:

J. Training Log Template

Training log V.1– 08/02/2023

Please describe your training and exercise schedule in as much detail as possible.

	Date:	Duration of session (min)	Average HR ($b.min^{-1}$)	Max HR ($b.min^{-1}$)	Activity (i.e., cycling/gym)	Weekly summated TSS (if available)
Week One						
Week two						
Week Three						
Week Four						
Week Five						
Week Six						
Week Seven						
Week Eight						

K. Athlete Wellbeing Questionnaire

Athlete Wellbeing Questionnaire V.1– 08/02/2023

Please rank the following wellbeing on a scale of 1-5.

	1	2	3	4	5	Record Score
Fatigue	Always tired	More tired than normal	Normal	Fresh	Very fresh	
Sleep Quality	Insomnia	Restless sleep	Difficulty falling asleep	Good	Very relaxed	
General Muscle Soreness	Very sore	Increase in soreness/tightness	Normal	Feeling good	Feeling great	
Stress Levels	Highly stressed	Feeling stressed	Normal	Relaxed	Very relaxed	
Mood	Highly annoyed/irritable	Snappiness towards friends/teammates	Less interested in others and/or activities than normal	A generally good mood	Very positive mood	

L. Illness Questionnaire

1.) Have you had any difficulties participating in any activities or performances due to health problems over the course of the trial?

- ❑ Full participation without any health problems
- ❑ Full participation, but with injury, illness, or health problem
- ❑ Reduced participation due to injury, illness or health problem
- ❑ Cannot participate due to injury, illness or health problem

2.) To what extent have you reduced the volume of your activities or performance over the trials?

- ❑ No reduction
- ❑ To a minor extent
- ❑ To a moderate extent
- ❑ Cannot participate at all

3.) To what extent has your health problems affected your performance during the trial?

- ❑ No effect
- ❑ To minor effect
- ❑ To moderate effect
- ❑ To major effect
- ❑ Cannot participate at all

4.) To what extent have you experienced health complaints during the trial's duration?

- ❑ No health complaints
- ❑ To a mild extent
- ❑ To a moderate extent
- ❑ To a severe extent

5.) If you have agreed to having any injuries or health problems, what are they?

- ❑ Injury
- ❑ Mental complaint
- ❑ Illness
- ❑ Other (e.g., operation or accident)