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IMPACT OF ACUTE FISH OIL OMEGA-3 POLYUNSATURATED FATTY ACIDS SUPPLEMENTATION ON ENDURANCE EXERCISE IN TRAINED CYCLISTS

This thesis was done under the mentorship of Dr Oliver Witard, PhD AFHEA, Senior Lecturer in Exercise Metabolism & Nutrition in the Faculty of Life Sciences and Medicine at King's College London, and Zvonimir Šatalić, PhD, Full professor at Faculty of Food Technology and Biotechnology at University of Zagreb, and with help of Niels Bootsma, PhD student from University of Stirling.

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IMPACT OF ACUTE FISH OIL OMEGA-3 POLYUNSATURATED FATTY ACIDS SUPPLEMENTATION ON ENDURANCE EXERCISE IN TRAINED CYCLISTS

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Abstract: Dietary supplementation with fish oil derived omega-3 polyunsaturated fatty acids (PUFAs) has been suggested to improve endurance exercise performance by enhancing oxygen supply to tissues, among other. Unlike long-term supplementation lasting one week or more, few studies have investigated the effects of acute supplementation with a single-dose of omega-3 PUFAs (e.g. 4.7 g/day) on performance outcomes by monitoring cardiovascular functions. This double-blind, randomised, crossover, placebo-controlled study investigated the acute effects of single-dose supplementation with omega-3 PUFAs from fish oil on endurance exercise performance in trained cyclists. It was hypothesized that there would be improvements in both exercise performance and efficiency, with the possibility that different forms of the supplements would have different effects. Seven participants (25,3±3,86 years) underwent laboratory testing. They consumed one of three supplement options (pre-emulsified, non-emulsified and placebo supplement) in random order before completing a 120-min submaximal pre-load cycling exercise to measure markers of exercise efficiency. Following a 5-min rest, participants completed a target workload time trial to measure endurance exercise performance. Heart rate, blood pressure and venous blood samples were collected during pre-load exercise. Blood pressure and blood samples for plasma fatty acid content were also taken before the exercise pre-load and post-time trial. Significant differences were observed between test supplements in omega-3 PUFA to total PUFA ratio, and for both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in the blood plasma ($p < 0.05$). Mean time trial completion time was not significantly different between interventions ($p > 0.05$). Mean heart rate, and systolic and diastolic blood pressure measured during the pre-load exercise also showed no significant differences between trials. This study did not find beneficial the use of a single-dose of omega-3 PUFAs for improvements in exercise efficiency or endurance performance in trained cyclists.

Keywords: *fish oil, omega-3 PUFAs, EPA, DHA, endurance exercise*

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UTJECAJ AKUTNE SUPLEMENTACIJE OMEGA-3 POLINEZASIĆENIM MASNIM KISELINAMA IZ RIBLJEG ULJA NA VJEŽBU IZDRŽLJIVOSTI KOD UTRENIRANIH BICIKLISTA

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Sažetak: Smatra se da bi suplementacija omega-3 polinezasićenim masnim kiselinama (PUFAs) iz ribljeg ulja mogla, među ostalim, povećati opskrbu tkiva kisikom i time poboljšati izvedbu u vježbama izdržljivosti. Za razliku od dugotrajne suplementacije, u trajanju jedan tjedan ili dulje, mnogo manje su istraženi učinci akutne suplementacije jednostrukom dozom omega-3 PUFAs (npr. 4.7 g/dan) na ishode treninga. Ova dvostruko slijepa, randomizirana, crossover, placebom kontrolirana studija istraživala je akutne učinke suplementacije jednostrukom dozom omega-3 PUFA iz ribljeg ulja na izvedbu treniranih biciklista. Pretpostavljeno je da će se izvedba i učinkovitost treninga izdržljivosti poboljšati, te da će prethodno emulgirani (pre-emulgirani) oblik suplementa imati bolje učinke od standardnog suplementa. Sedam ispitanika (25,3±3,86 godina) sudjelovalo je u laboratorijskim testiranjima. Ispitanici su konzumirali jednu od tri vrste suplementa (pre-emulgirani, standardni i placebo) nasumičnim redoslijedom prije svake 120-minutne vožnje bicikla pri submaksimalom opterećenju kako bi se izmjerili pokazatelji učinkovitosti vježbanja. Nakon 5-minutnog odmora ispitanici su bili podvrgnuti testiranju pri ciljanom opterećenju u ograničenom vremenu zbog mjerenja učinaka suplementacije na tjelesnu izdržljivost. Puls, krvni tlak, te uzorci krvi za određivanje sadržaja masnih kiselina u plazmi uzeti su prije i tijekom 120-minutne vožnje bicikla i nakon testiranja. Uočene su značajne razlike između ispitivanih suplemenata u omjeru omega-3 PUFAs i ukupnih PUFAs, te u razinama eikozapentaenske (EPA) i dokozaheksaenske kiseline (DHA) u krvnoj plazmi ($p < 0,05$). Prosječno vrijeme trajanja testiranja pri ciljanom opterećenju nije se značajno razlikovalo obzirom na konzumirani suplement ($p > 0,05$). Prosječni broj otkucaja srca, te sistolički i dijastolički krvni tlak mjereni tijekom bicikliranja pri submaksimalnom opterećenju također nisu pokazali značajne razlike između testiranja. Rezultati ovog istraživanja nisu potvrdili da bi jednokratna doza omega-3 PUFAs bila korisna za poboljšanje učinkovitosti vježbanja ili izdržljivosti kod utreniranih biciklista.

Ključne riječi: riblje ulje, omega-3 PUFAs, EPA, DHA, vježba izdržljivosti
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1. INTRODUCTION

Omega-3 polyunsaturated fatty acids (PUFAs) are group of essential fatty acids for humans which have to be obtained through diet (Hamazaki et al., 1996). Alpha-linolenic acid (ALA) is the simplest form of omega-3 PUFAs. It can be converted by enzymatic reactions of elongation and desaturation into two longer chain omega-3 PUFAs: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, human production of those longer chain omega-3 PUFAs from ALA is limited, so it is necessary to incorporate sources of EPA and DHA into the diet (Shahidi and Ambigaipalan, 2018). Most common dietary sources of preformed EPA and DHA are oily fish (i.e. body lipids of fatty fish, the liver of white lean fish, and the blubber of marine mammals) and fish oil supplements (Shahidi and Ambigaipalan, 2018; Purcell et al., 2014).

Over the past several decades the interest in the effects and benefits of dietary omega-3 PUFAs on human health has raised. It was revealed that those essential fatty acids influence many physiological and pathophysiological aspects of the human body (Hamazaki et al., 1996) The omega-3 PUFAs effects on human health are mainly from their anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic and antiproliferative properties (Da Boit et al., 2017). Since omega-3 PUFAs are constituents of cell membranes, they contribute to the optimal structure, function, and responses of cells within the body (Da Boit et al., 2015). This consequently affects physico-chemical properties of the cells and, ultimately, organ functions (Allaire et al., 2017) and can play a major part in the treatment and prevention of many conditions related to poor health and wellbeing (Calder, 2012). The mechanisms of action of omega-3 fatty PUFAs from fish oil are complex and still not completely understood.

Western diets are poor in the amount of omega-3 PUFAs consumed (Walker et al., 2015). Studies have shown that dietary supplementation with omega-3 PUFAs modulate a variety of cardiometabolic risk factors and leads to improvements in cardiac functions in humans (Kawabata et al., 2014; Calder, 2012).

Regarding physical activity and exercise, dietary factors play an important role in optimizing the training process in athletes and many athletes use dietary supplements. Because of the aforementioned properties, fish oil omega-3 PUFAs have been recommend for athletic population (Da Boit et al., 2015). Moreover, omega-3 PUFAs are one of the most popular dietary supplements used by athletes (Philpott et al., 2018). Omega-3 PUFAs have been

showing potential for improvements in physical performance (Da Boit et al., 2017; Peoples and McLennan, 2016). One possible mechanism in which they might improve exercise performance is by positive impact on vascular function (Zebrowska et al., 2015). In theory, the omega-3 PUFAs could improve vascular blood flow during exercise by increase vasodilation through their anti-inflammatory properties. That could lead to increment in oxygen supply to tissues or reduce oxygen consumption without changes in tissue work (Kawabata et al., 2014).

Many previous studies have focused on long-term supplementation of omega-3 PUFAs to determine its health benefits and effects on exercise performance (Allaire et al., 2017; Da Boit et al., 2017; Peoples and McLennan, 2016). However, there are very few studies using a single dose supplementation with omega-3 PUFAs to investigate its acute effects and impact on endurance performance, especially in a trained population (Rontoyanni et al., 2012). To examine the acute effects of a supplement, it is important to achieve its rapid action in human organism. Considering that the form of the fish oil supplements determines their bioavailability and absorption by the human body, the process of pre-emulsification of omega-3 PUFAs supplements has been theorized as beneficial for the improvement of the absorption rates (Walker et al., 2015; Garaiova et al., 2007).

The purpose of this study was to determine the impact of acute supplementation with omega-3 PUFAs from fish oil on endurance exercise performance in trained cyclists, and to investigate acute changes in indirect markers of exercise efficiency (i.e. heart rate and blood pressure). It was hypothesized that a single-dose of fish oil supplement would lead to an improvement in both exercise performance and efficiency, with the possibility that the pre-emulsified form of the supplement would have a more favorable effects than the non-emulsified, standard supplement.

2. THEORETICAL PART

2.1. DIETARY LIPIDS

Lipids are a large group of structurally and functionally diverse but similar chemical compounds of natural origin, which occupy an important role in human nutrition (Thompson, 2020; Kritchevsky, 2008; O'Keefe, 2008). Some of the major lipid subgroups are fatty acids, acylglycerols, sterols, waxes, phospholipids, and others. One of the main characteristics of lipids is their insolubility in water but solubility in organic, nonpolar solvents, due to presence of hydrophobic hydrocarbon chain in their structure (O'Keefe, 2008).

These unique biological molecules are one of the most important components of natural foods, and have important functions in higher life forms (Režek Jambrak and Škevin, 2017). Alongside carbohydrates and proteins, lipids are one of the main structural components of living cells. Also, they are a source of energy and therefore important components of the human diet (German, 2011). Energy value of lipids is 9 kcal per gram which is more than twice as per gram of carbohydrates and proteins (Sanders 2016). Lipids have a broad range of functions in living organisms. At the cellular level, lipids are critical for cell structure and function, they serve as a signaling molecules and provide substances that regulate physiological processes, as well as storage of energy for the whole organism, and body insulation and protection (Amin et al., 2019; German, 2011; Kritchevsky, 2008). Various studies have shown that lipids play an important role in human health in relation to cardiovascular diseases, inflammation, cancer, diabetes mellitus, obesity, and neuropsychiatric disorders (Li and Sinclair, 2002).

Based on their composition and physical properties of lipids at room temperature, we distinguish liquid oils and solid fats. General differences between oils and fats derived from different sources (plant, animals and microorganisms) are in their constituents (Thompson, 2020; Režek Jambrak and Škevin, 2017). Most dietary lipids consist of free fatty acids and triglycerides (TGs) or triacylglycerols (TAGs), which are esters of glycerol and three fatty acids (Domínguez-Avila and González-Aguilar, 2018). The molecule of triglyceride is composed of hydrocarbon chain which has three carbon atoms with hydroxyl (–OH) group on each. That makes a glycerol molecule. Each hydroxyl (–OH) group of glycerol is linked to a fatty acid. In general, fatty acids (FAs) are hydrocarbon chains with an even number of carbon

atoms and a carboxyl group (–COOH) at one end (Jones and Rideout, 2012). All chemical reactions take place on fatty acids what makes them the most important part of the triglyceride molecule.

2.2. FATTY ACIDS

Differences in fatty acids structure are the reason for their functional diversity and distinctive impacts on human health (Vannice and Rasmussen, 2014; German, 2011). Fatty acids are classified by both hydrocarbon chain length, depending on the number of carbon atoms in individual fatty acids structure, and their saturation status, that is fatty acids differ in the number and position of double bonds along the hydrocarbon chain (Jones and Rideout, 2012).

The length of the carbon chain in dietary fatty acids may vary from 4 to 26 carbon atoms, but mostly contain 12 to 22 carbons (Vannice and Rasmussen, 2014). Their structures can be modified by enzyme desaturation and chain elongation reactions to produce a variety of metabolites with individual chemical and physical properties (Kritchevsky, 2008).

Fatty acids are often categorized into short chain (up to 6 carbons), medium chain (8 to 12 carbons), or long chain (more than 12 carbons) (Vannice and Rasmussen, 2014).

Also, fatty acids are classified by their varying degrees of saturation, depending on the presence and position of double bonds in carbon chain (Vannice and Rasmussen, 2014; Jones and Rideout, 2012). The main division is into saturated (SFAs) with no double bonds, and unsaturated fatty acids (UFAs) with one or more double bonds. The main subgroups of UFAs are monounsaturated fatty acids (MUFAs) which have one double bond, and polyunsaturated fatty acids (PUFAs) with two or more double bonds (Jeromson et al., 2015).

As mentioned previously, the differences in the chemical structure of fatty acids are associated with their different physiological effects in human organism. For example, SFAs has been linked with the development of metabolic dysfunction while for some MUFAs and PUFAs is believed to have positive effects on metabolic function (Jeromson et al., 2015; Vannice and Rasmussen, 2014).

Almost all of the unsaturated fatty acids consumed in human diets are members of the n-3, n-6, n-7, or n-9 families of fatty acids (Watkins and German, 2008).

During the biosynthesis of fatty acids human enzymes can form the first double bond at ninth carbon (n-9) from the methyl end in the carbon chain. Methyl end is also called the omega end, and hence fatty acids with double bonds in their structure are called omega fatty acids (Jones and Rideout, 2012). The marking "a:b" is used to denote the chain length by number of carbon atoms (a) and number of double bonds in the FA structure (b), and the notation "n:x" or "ω:x" are used to describe the position of the double bond nearest to the methyl end. This is the so-called omega- or n-system of numbering and designation of fatty acids, which starts at the methyl end of the fatty acid (Figure 1). 5 san used when fatty acids are discussed regarding to nutrition, simplifying investigation into the nutritional relevance of fatty acids (Watkins and German, 2008).

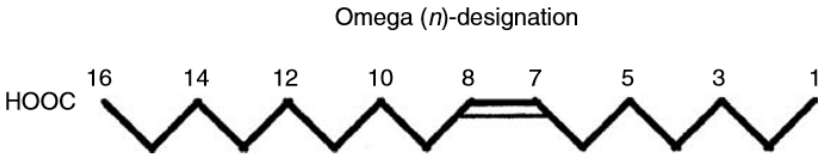


Figure 1. The omega- or n-numbering system for palmitoleic acid (16:1, n-7). n-numbering starts at the methyl terminus carbon (Watkins and German, 2008)

Fatty acids with double bonds at closer positions than n-9, for example at the n-3 and n-6 positions, are considered to be essential in the human diet (Jones and Rideout, 2012).

All groups of unsaturated fatty acids are metabolized by the same set of enzymes to their particular metabolites (Das, 2006).

2.2.1. Polyunsaturated fatty acids (PUFAs)

Polyunsaturated fatty acids (PUFAs) are characterized by the content of two or more double bonds in the main carbon chain of the molecule. They are in a liquid state at room temperature and are often referred to as oils. In a last few decades, researchers have discovered that the function of PUFAs in human nutrition differ due to their structure (Vannice and Rasmussen, 2014). PUFAs can be classified based on the position of the first double bond on the fatty acid carbon chain, precisely how far is the double bond from the terminal methyl group (i.e. methyl end) of fatty acid. Each subsequent double bond takes

place three carbon atoms farther along the carbon chain from the bond preceding it. Therefore, the number of double bonds in single fatty acid is restricted depending on its chain length, but it will not exceed six (Jones and Rideout, 2012). Longer chain (20 or more carbon atoms) PUFAs are sometimes called highly unsaturated fatty acids (HUFAs) (Vannice and Rasmussen, 2014).

2.2.2. Essential fatty acids (EFAs)

One of the principal roles of fat in the human diet is the provision of the essential fatty acids and their derivatives (Sanders, 2016). The essentiality of fatty acids in human nutrition depends on the position of the first double bond from the methyl end in the carbon chain (Jones and Rideout, 2012). All essential fatty acids, which originate from the families of n-3 or n-6 fatty acids, are polyunsaturated and their main characteristic is the presence of two or more cis- double bonds (Klurfeld, 2008).

In the n-3 fatty acids the first double bond occurs at the third carbon atom from the methyl end of the carbon chain, while the n-6 fatty acids have the first double bond at the sixth carbon atom from the methyl end (Vannice and Rasmussen, 2014).

Although some desaturase enzymes naturally exist in the human body, we are lacking those desaturases specific for the de novo synthesis of n-3 and n-6 fatty acids (Kritchevsky, 2008; Jones and Rideout, 2012). However, some n-3 and n-6 fatty acids could be synthesized in humans and other mammals by desaturation and elongation reactions of the dietary precursors. This series of a linked reactions primarily take place in the liver (Calder, 2012; Jones and Rideout, 2012).

The n-3, n-6, and n-9 fatty acids compete with each other for the desaturase and the elongase enzymes involved in the PUFA formation. Because of this competitive nature of fatty acid desaturation and elongation, each class of essential fatty acids can interfere with the metabolism of the other. This competition has nutritional implications because the efficiency of PUFA synthesis is limited, and could possibly lead to a deficiency of essential fatty acids and their metabolites (Hernandez, 2016; Calder, 2012; Jones and Rideout, 2012; O'Keefe, 2008). For that reason n-3 and n-6 PUFAs are considered essential nutrients for human organisms and humans depend on dietary intake of PUFAs that are synthesized by other organisms (i.e. plants and animals) (Hernandez, 2016; Vannice and Rasmussen, 2014).

Insufficient intake of essential fatty acids has expansive implications for development and the lifelong health of humans (German, 2011).

Two groups of fatty acids essential for humans are the n-6 derived from parental linoleic acid (LA, 18:2, n-6) and the n-3 derived from parental α-linolenic acid (ALA, 18:3, n-3). In order to achieve their maximum benefits, they need to be metabolized to their long-chain metabolites (Das, 2006).

Some of the functions of EFAs require their conversion to eicosanoids and other products, in most cases the fatty acids themselves appear to be biologically active (Das, 2006).

Whether the double bond is located on carbon number 3 or 6 (as in n-3 and n-6 PUFA) makes a significant difference in their biological function (Vannice and Rasmussen, 2014).

It is the position of the Academy of Nutrition and Dietetics (the Academy) that dietary fat for the healthy adult population should provide 20% to 35% of energy, with an increased consumption of n-3 polyunsaturated fatty acids (Vannice and Rasmussen, 2014).

2.2.2.1. Omega-6 (n-6) fatty acids

In the omega-6 (n-6) family of PUFAs linoleic acid (LA, 18:2, n-6) (Figure 2) serves as a precursor for the production of the series of metabolites (Watkins and German, 2008; Kritchevsky, 2008).



Figure 2. Chemical structure of linoleic acid (Marventano et al., 2015)

Plant organisms have the ability to synthesize LA, whilst animals, including humas, are incapable of producing it *de novo* (Watkins and German, 2008). Linoleic acid itself has a physiological structural role in cell membranes (Sanders, 2016).

In humans, LA is converted to γ-linolenic acid (GLA, 18:3, n-6) by the action of the enzyme desaturase, and GLA is further metabolized to form dihomo-γ-linolenic acid (DGLA, 20:3, n-6). DGLA is the elongation product of linoleic acid conversion, and serves as a precursor for the formation of the arachidonic acid (AA, 20:4, n-6) (Figure 3) and some other metabolites of n-6 acyl species with potential therapeutic effects (Watkins and German, 2008; Das, 2006). Arachidonic acid, as the product of desaturation and elongation of linoleic acid (Figure 2), is considered as an essential because of its action as the precursor for the production of eicosanoids, which regulate many physiological processes (Sanders, 2016; Kritchevsky, 2008).



Figure 3. Chemical structure of arachidonic acid (Marventano et al., 2015)

2.2.2.2. Omega-3 (n-3) fatty acids

The omega-3 (n-3 or ω-3) PUFAs are family of biologically active compounds, derived from an essential α-linolenic acid (Calder, 2012; Kritchevsky, 2008). Omega-3 PUFAs include α-linolenic acid (ALA, 18:3, n-3), stearidonic acid (SDA, 18:4, n-3), eicosapentaenoic acid (EPA, 20:5, n-3), docosapentaenoic acid (DPA, 22:5, n-3), and docosahexaenoic acid (DHA, 22:6, n-3) (Shahidi and Ambigaipalan, 2018). Although it is the simplest omega-3 fatty acid, α-linolenic acid (ALA,18:3, n-3) serves as the metabolic precursor for the production of other n-3 fatty acids in animals, especially eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3) (Figure 4) (Watkins and German, 2008; Das, 2006). EPA and DHA are thought to be the most bioactive of the omega-3 family (Jeromson et al., 2015).

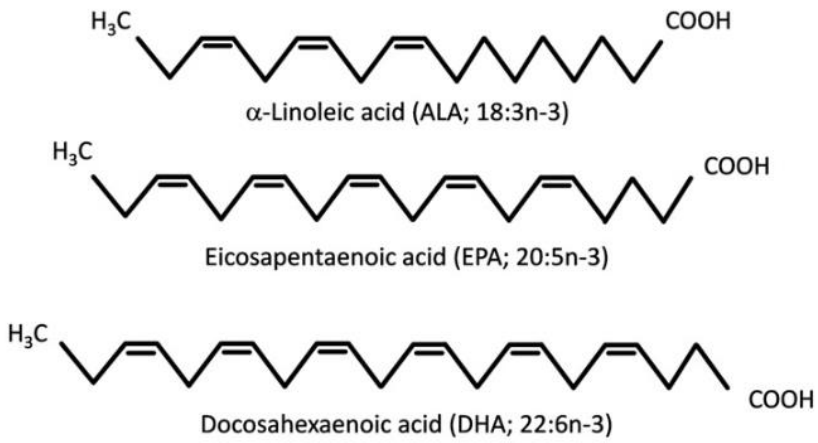


Figure 4. Chemical structures of omega-3 fatty acids: α-linolenic acid, eicosapentaenoic and docosahexaenoic acid (Marventano et al., 2015)

Unlike plants, humans do not possess the enzyme needed for synthesis of ALA, but they can metabolize it to longer chain, more unsaturated and more biologically active n-3 fatty acids by processes of chain elongation, desaturation, β-oxidation, etc. (Calder, 2012; Kritchevsky, 2008).

α-Linolenic acid can be desaturated to stearidonic acid (SDA, 18:4, n-3), which is an intermediate in the metabolic pathway of ALA (Calder, 2012; Watkins and German, 2008). Its further conversion yields eicosapentaenoic acid (EPA, 20:5, n-3) (Calder, 2012). Conversion of ALA to EPA competes with the conversion of LA to AA, because the same enzymes are used in these reactions (Calder, 2012). In other words, EPA acts as a competitive inhibitor of arachidonic acid metabolism (Kritchevsky, 2008). By further elongation, EPA can be converted to docosapentaenoic acid (DPA, 22:5, n-3) which is a precursor for the production of very long-chain unsaturated fatty acid, docosahexaenoic acid (DHA, 22:6, n-3). It has also been reported that EPA production can occur in animals by the β-oxidation chain shortening of DHA (Kritchevsky, 2008).

DHA and EPA are the most important omega-3 PUFAs for humans. They should be synthesized from essential fatty acid in (ALA) or taken directly preformed in the diet (Drobnic et al., 2017). Biological conversion of ALA to EPA, DPA, and DHA in human organism was demonstrated to be generally poor. It is much more useful for humans to ingest preformed EPA and DHA from diet, rather than ALA (Drobnic et al., 2017; Walker et al., 2015; Calder, 2012; Jones and Rideout, 2012).

2.2.3. Dietary sources of essential fatty acids

Food of plant origin and food of animal origin differ in total quantity of fats and their metabolites, its tissue distribution, and the composition of storage fat. These could be significant nutritional, biochemical, and physiological differences. Thus, it is advisable for humans to consume both types of food (Watkins and German, 2008).

Unlike plants, animals are incapable of synthesizing linoleic and α-linolenic acids so modern agriculture has greatly enriched food for livestock with those fatty acids, especially linoleic acid. In addition to that, n-6-rich crops are more stable than n-3-rich crops, so their cultivation and use as food ingredients is preferred (Watkins and German, 2008; Das, 2006). As a result, a significant shift in the natural balance of LA and ALA has been observed (Kritchevsky, 2008). The average dietary consumption of ALA has declined significantly, and humans nowadays consume a large amounts of LA, especially from meats (Kritchevsky, 2008; Watkins and German, 2008).

The major source of EFAs in human diet are LA and ALA from plants, and EPA and DHA from marine fish and seafood (Vannice and Rasmussen, 2014). LA and ALA are the two primary PUFA products of plant fatty acid biosynthesis (Watkins and German, 2008).

The plant-based food is abundant with LA, while the distribution and abundance of ALA are more limited (Sanders, 2016). The main dietary sources of LA are cereals and vegetable oils like sunflower, saffola, and corn oil. ALA is found in significant amounts in walnuts, flaxseed, chia and hemp seeds, dark green leafy vegetables and vegetable oils, such as canola, rapeseed and soybean oils (Sanders, 2016; Vannice and Rasmussen, 2014; Das, 2006).

Evening primrose oil, borage oil, black currant oil, and hemp seed oil contain substantial amounts of GLA (Sanders, 2016; Das, 2006). SDA is not typically found in food, very small amounts occur in fish and some plant sources (e.g., echium, black currant) (Vannice and Rasmussen, 2014). AA, EPA and DHA are produced in notably amounts in marine algae (Watkins and German, 2008).

In most animals long-chain, highly unsaturated fatty acids are not esterified into triacylglycerols. However, fish can accumulate PUFAs, substantial amounts of the EPA and DHA, but only if they or their precursors are present in the fish diet. Fish actually have dietary requirements for n-3 PUFAs but are unable to synthesize them (Sanders, 2016; Watkins and German, 2008; Das, 2006). Despite that, EPA and DHA are considered the primary fatty acids

of fish oil (Hernandez, 2016; Purcell et al., 2014; Kritchevsky, 2008). They are commonly called omega-3 fish oils.

Lean fish store lipid in their liver (e.g. cod), and oily fish store lipid in their flesh (e.g. mackerel, herring, salmon, tuna, sardines) (Calder, 2012). EPA and DHA also occur in the blubber of some marine mammals (Shahidi and Ambigaipalan, 2018). Fresh water fish are unlikely to contain notable amounts of EPA and DHA (Das, 2006). The best sources of preformed EPA, DPA, and DHA is fish like salmon, anchovy, sardines, cod, tuna, herring, and trout (Vannice and Rasmussen, 2014; Purcell et al., 2014). Therefore, consumption of fatty fish and fish oil can significantly contribute to long-chain omega-3 PUFA intake (Sanders, 2016).

2.2.4. Digestion of omega-3 PUFAs

The omega-3 PUFAs may be present in food and supplements as ethyl esters (EEs), TAGs, free fatty acids (FFAs), or phospholipids (PLs). The form in which they exist influence their bioavailability (Shahidi and Ambigaipalan, 2018). Unlike other macronutrients, the hydrophobic nature of lipids requires specialized processes during their metabolism. Digestion of dietary lipids involves a series of specific processes enabling absorption through the hydrophilic environment of the gut (Jones and Rideout, 2012).

The oral cavity is the starting point of fat digestion. After the processes of salivation and mastication, enzyme lingual lipase from the serous glands of the tongue initiates the degradation of fat molecules (Jones and Rideout, 2012; Garaiova et al., 2007). The digestion continues into the stomach, where physical mixing action and enzymes gastric lipases continue to break down lipids, and form emulsions of fat globules (Shahidi and Ambigaipalan, 2018; Jones and Rideout, 2012; Garaiova et al., 2007). Emulsified fat globules are mainly consisted of TAGs but also free fatty acids (FFA) and diacylglycerols (Garaiova et al., 2007). The final and complete digestion of formed fat emulsion happens in the intestinal lumen by actions of bile salts and pancreatic lipases, the principal enzymes of TG digestion which hydrolyze ester bonds (Shahidi and Ambigaipalan, 2018; Jones and Rideout, 2012). FAs and MAGs are formed, they are incorporated into mixed micelles and their absorption into the enterocytes occurs by passive diffusion (Shahidi and Ambigaipalan, 2018; Garaiova et al., 2007).

However, dietary omega-3 fatty acids pass through the stomach into the small intestine, where their digestion is continued (Figure 5) (Shahidi and Ambigaipalan, 2018; Jones and Rideout, 2012).

Research has shown that digestion and absorption of different forms of ω-3s (e.g. EEs, TAGs, or PLs) is enhanced with the higher lipid content of the meal, because it increases the activity of pancreatic enzymes. Conversely, digestion and absorption of free forms of ω-3 PUFAs have been shown not to depend of pancreatic enzymes and meal lipid content (Shahidi and Ambigaipalan, 2018).

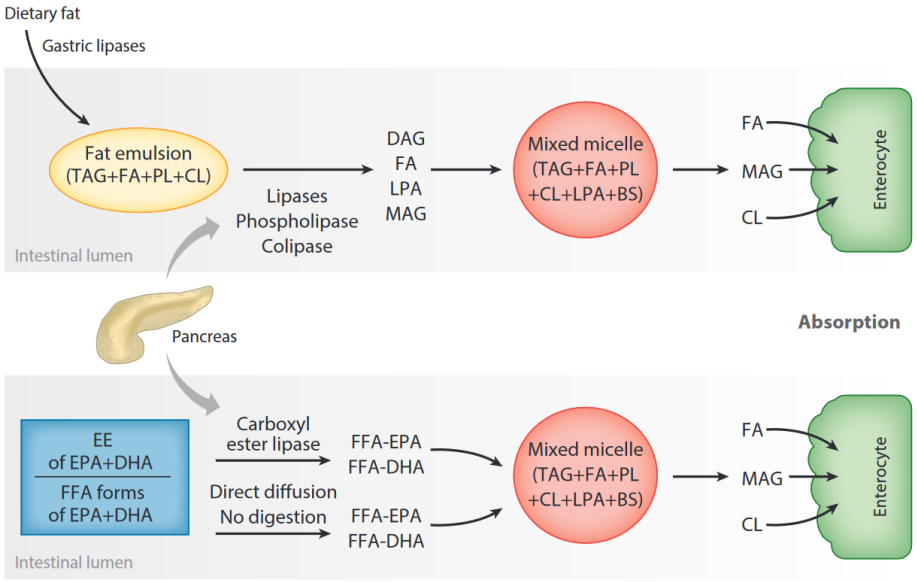


Figure 5. A schematic display of dietary fat digestion and absorption of free fatty acid (FFA) forms of ω-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

Abbreviations: BS - bile salt, CL - cholesterol, DAG - diacylglycerol, FA - fatty acid, LPA - lysophosphatidic acid, MAG - monoacylglycerol, PL - phospholipid, TAG - triacylglycerol

(Shahidi and Ambigaipalan, 2018)

Inside the enterocyte, long-chain fatty acids (with chain length greater than 12 carbon atoms) are re-synthesized into TAG and incorporated into the chylomicrons (CM), a type of aggregates of lipids and proteins (i.e. lipoproteins). In this manner, hydrophobic lipids can be secreted and transported within the aqueous media of lymphatic system, from where they enter the circulation (Jones and Rideout, 2012; Garaiova et al., 2007). Depending on the needs of the organism, chylomicrons will allocate to the body cells through blood circulation.

Liver is the site of most of the PUFA metabolism that transforms dietary C18 EFAs into 20–22C PUFAs. Long-chain PUFAs are then transported to extrahepatic tissues by chylomicrons.

Then, the enzymatic hydrolysis of chylomicrons by lipoprotein lipase occurs at the surface of tissues. Released fatty acids and TAGs are taken up into the cells and chylomicron remnants are removed from the blood plasma in the liver by low density lipoproteins (LDL) (Jones and Rideout, 2012; Garaiova et al., 2007).

The transport of fatty acids into the cells must be precisely regulated because high intracellular levels of free fatty acids can be highly toxic due to lipid peroxidation. Highly unsaturated fatty acids, like omega-3 PUFAs, will be oxidized the fastest. There are many transporter proteins and cytosolic proteins that regulate uptake and subcellular localization of fatty acids, allowing for them to be stored or metabolized effectively (Jeromson et al., 2015).

After fatty acids enter the cells, they are directed towards mitochondria if they need to be used in energy metabolism. When fatty acids enter the mitochondria, they are broken down in a series of reactions called β-oxidation to numerous acetyl-CoA and H^+ molecules, which are further used in energy metabolism (Hunter, 2012).

Uptake and further metabolism of PUFAs differ among various tissues. The final tissue composition of long-chain PUFAs is an outcome of the foregoing complex processes and influence of dietary factors (Jones and Rideout, 2012).

2.2.5. Functions of Essential omega-3 Fatty Acids

Many epidemiological and clinical studies have shown the benefits of diverse physiological effects of omega-3 PUFA on human health. Essential fatty acids, particularly EPA and DHA as the most important omega-3 PUFAs, are necessary for the normal functioning of all tissues (Režek Jambrak and Škevin, 2017; Jones and Rideout, 2012; Jump, 2002).

In general, intake of EPA and DHA will result in their distribution to the various body cells and will have impact on membrane structure and function, synthesis and activity of eicosanoids, and the regulation of gene expression. (Das, 2006; Jump, 2002).

2.2.5.1. Effects of Omega-3 PUFAs on Cell Membrane Structure and Function

Even small changes in biochemical properties of cell phospholipid membrane are sufficient to cause altered functioning of the cells (Režek Jambrak and Škevin, 2017; Bortolotti et al., 2007; Das, 2006). The fluidity of the cell membrane is determined by its lipid composition. Cell membranes are more rigid when composed of saturated fatty acids and cholesterol molecules. Increased incorporation of unsaturated fatty acids into the cell membrane phospholipid bilayer affords a more structure (Das, 2006). Changes in cell membrane composition can, in turn, affect membrane order, insulin sensitivity, intracellular signaling processes, gene expression, and the production of lipid and protein mediators as a response to injury and inflammatory processes (Calder, 2012; Das, 2006).

Incorporated EFAs can be released from cell membrane phospholipids, and then transformed into intracellular metabolites (e.g. diacylglycerol) and extracellular metabolites (e.g. eicosanoids), which participate in important cell signaling (Jones and Rideout, 2012).

Furthermore, as rigidity of the body cell membranes increases, the number of insulin receptors on top of the cells decreases, as well as their affinity to bind insulin molecules. This can result in insulin resistance of the cell. Otherwise, increase in cell membrane fluidity increases the number of insulin receptors on it and their affinity to insulin enhances. This reduces insulin resistance, and improves insulin sensitivity, respectively (Das, 2006). These changes impact the way cells interact with their surroundings and have great effect on glucose and lipid metabolism in human body (Albert et al, 2014; Jump, 2002).

Red blood cell deformability refers to changes in their shape formed while passing through the microcirculation. This is associated with increased aggregation of red blood cells in the endothelium, and consequently causes lower oxygen delivery through the body. Although dietary fish oil increases the deformability of red blood cells, as a consequence of incorporation of omega-3 PUFAs into their membrane phospholipids, it also facilitates the transport of red blood cells through the capillaries. That could lead to enhanced oxygen delivery. EPA has been incorporated more efficiently into red blood cell membranes than DHA (Mickelborough, 2013).

2.2.5.2. Effects of Omega-3 PUFAs on Gene Expression

Omega-3 PUFAs, EPA and DHA, have significant effects on the regulation and expression of specific genes connected to glucose and lipid metabolism (Gani, 2008; Bortolotti et al., 2007; Jump, 2002). This activity of EPA and DHA increases oxidation and uptake of fatty acids and their metabolites, and also enhances storage of fatty acids in the adipose tissue. The availability of fatty acids for energy production is reduced, which leads to increased utilization of glucose than fatty acids as a fuel in the skeletal muscles. Insulin sensitivity is enhanced, and the body can manage glucose and fatty acids more effectively (Albert et al, 2014; Gani, 2008). In addition, EPA and DHA modify the gene expression for inflammatory species, like eicosanoids and cytokines, and by that decreasing their production (Mickleborough, 2013).

2.2.5.3. Effects of Omega-3 PUFAs on synthesis and activity of eicosanoids

PUFAs with chain length of at least 20 carbon atoms, are susceptible to oxidation and can be converted into the eicosanoids by enzymatic and nonenzymatic pathways (Markworth et al., 2013; Jones and Rideout, 2012; Das, 2006). Thus, EFAs are the precursors for eicosanoids, a large group of lipid metabolites with very potent biological activities (Klurfeld, 2008; Kritchevsky, 2008). Some subtypes of eicosanoids include the prostaglandins, thromboxanes, prostacyclins, leukotrienes, anandamides, hydroperoxytetraenoic acid, hydroxy-eicosatetraenoic acid, lipoxins and resolvins (Shahidi and Ambigaipalan, 2018; Sanders, 2016; Calder, 2012).

Eicosanoids regulate many physiological processes in humans (Calder, 2012; Jones and Rideout, 2012). However, the physiological functions of eicosanoids depend on which precursor they are synthesized from. There are differences between ones derived from arachidonic acid and those derived from EPA and DHA (Shahidi and Ambigaipalan, 2018). Humans depend on the dietary intake of the essential omega-3 and omega-6 PUFAs for adequate biosynthesis of eicosanoids (Jones and Rideout, 2012). Major eicosanoids involved in physiology and pathophysiology are synthesized from arachidonic acid, and the ones produced from EPA and DHA usually have opposite properties and actions (Calder, 2012; Jones and Rideout, 2012).

An imbalance between these two types of eicosanoids, in terms of overproduction of arachidonic acid eicosanoids, has been proposed to contribute to pathologic changes and development of disease processes (e.g. disorders like thrombosis, vasoconstriction, arthritis, lupus nephritis, psoriasis, asthma, and inflammatory bowel disease) (Shahidi and Ambigaipalan, 2018; Jones and Rideout, 2012).

High dietary intake of EPA and DHA reduces the availability of arachidonic acid in the organism as a substrate for eicosanoid biosynthesis (Calder, 2012). This consequently modifies the pattern of production of eicosanoids, by inhibiting their production, and also inhibits arachidonic acid metabolism (Calder, 2012; Jones and Rideout, 2012).

EPA, for example, is a much less preferred substrate for both pathways compared with arachidonic acid for production of eicosanoids, and by substrate competition, EPA inhibits release of arachidonic acid derived eicosanoids (Mickleborough et al., 2013). In this way omega-3 PUFAs can affect the actions regulated by arachidonic acid eicosanoids (Calder, 2012).

It is considered that omega-3 EFAs possess anti-inflammatory properties due to production of eicosanoids with beneficial actions on pathologic processes (Jones and Rideout, 2012; Das, 2006). Eicosanoids modulate cardiovascular, pulmonary, immune, reproductive, and secretory functions in many cells (Jones and Rideout, 2012). They play a crucial role in cardiovascular physiology by being involved in pro- and anti-inflammatory actions, pro- and anti-platelet aggregatory actions, vasodilation, vasoconstriction, immune responses, cell growth and proliferation (Shahidi and Ambigaipalan, 2018). Various scientific studies found that omega-3 PUFA derived eicosanoids can improve cardiovascular function due to their anti-inflammatory and anti-thrombotic properties. Namely, they can beneficially modify many risk factors for cardiovascular diseases (e.g. blood pressure, platelet reactivity and thrombosis, plasma TG concentrations, vascular function, cardiac arrhythmias, heart rate variability) (Režek Jambrak and Škevin, 2017; Calder, 2012; Das, 2006; Jump, 2002).

2.2.6. General health benefits of omega-3 fatty acids

Considering a variety of biological actions of omega-3 PUFAs, they can have a significant role in health promotion and reduction of disease risk, and are essential for normal growth and development (Režek Jambrak and Škevin, 2017; Calder, 2012; Jump, 2002).

The effect of omega-3 fatty acids on cardiovascular health is one of the most studied areas of nutrition science (Vannice and Rasmussen, 2014). Significantly reduced morbidity and mortality from cardiovascular diseases are associated with increased intake of very long-chain omega-3 PUFAs (Calder, 2012). Scientific evidences suggest that omega-3 EFAs consumption can reduce inflammation processes, improve endothelial function, normalize heart rate variability, improve myocardial relaxation and efficiency, lower both systolic and diastolic blood pressure, and limit platelet aggregation (Shahidi and Ambigaipalan, 2018; Vannice and Rasmussen, 2014; Das, 2006; Li and Sinclair, 2002). The cardioprotective effects of omega-3 PUFAs have been linked exactly to substrate competition between essential omega-3 PUFAs and arachidonic acid for cyclooxygenase enzymes, which are responsible for production of eicosanoids (Shahidi and Ambigaipalan, 2018).

Moreover, omega-3 PUFAs decrease the production of inflammatory eicosanoids, cytokines, and reactive oxygen species, they also have immunomodulatory effect (Mickleborough, 2013).

Several studies have shown beneficial effects of omega-3 PUFAs or fish oil supplementation against type 2 diabetes, given that regular consumption of omega-3 PUFAs in diet could increase insulin sensitivity (Shahidi and Ambigaipalan, 2018; Gao et al., 2017).

Both experimental and epidemiological studies have shown that omega-3 PUFAs reduce the risk of cancer due to their anticarcinogenic effect (Shahidi and Ambigaipalan, 2018). Supplementation with omega-3 PUFAs can prevent neurotoxicity in addition to cancer treatment and improve the efficacy and tolerability of chemotherapy, as well (Shahidi and Ambigaipalan, 2018).

Furthermore, omega-3 PUFAs are of great importance for normal cognitive, neurological and visual development and function, especially during the perinatal period and adolescence. The reason is that DHA is a essential component of cell membrane phospholipids in human brain and retina (Shahidi and Ambigaipalan, 2018; Da Boit et al., 2017; Hernandez, 2016; Sanders, 2016; Das, 2006). Also, omega-3 can be beneficial in prevention of neurodegenerative diseases (Calder, 2012).

Overall, omega-3 PUFAs have a broad variety of biological actions beneficial for the human health (e.g. anti-aggregatory, anti-inflammatory, antiproliferative, antiarrhythmic, antiatherogenic, immunosuppressive, anti-bacterial, anti-viral and anti-fungal actions). Most likely their effects on health are dose dependent (Hernandez, 2016; Calder, 2012; Simopoulos,

2007; Das, 2006). An adequate intake for adults in general, recommended by European Food Safety Authority, for both EPA and DHA is 250 mg per day (EFSA, 2019).

2.3. ENERGY METABOLISM AND EXERCISE

Multiple body systems interact during complex physical activity, and by repeating it consequently they physiologically adapt which results in improvement of their functions. In sport, improvements in body systems functions means improvements in athletes' performance. In that case, even minor advantages in any area can have significant impacts on results.

For athletes, nutrition is one of the key interventions in the process of improving their sports performance. Therefore, main objectives in sports nutrition are as follows: promotion of good health, adaptations to training, quick recovery after trainings, and optimal performance during competitions (Peoples and McLennan, 2016; Williams, 2012).

The availability of energy substrates to the muscle cells, is important aspect of exercise performance because energy production in the muscles enables body movement (Da Boit et al., 2017). Various forms of energy stores in muscles contribute to production of energy, and which one will be used depends primarily on the intensity of exercise (Williams, 2012). Adenosine triphosphate (ATP) is instant source of energy for high-intensity exercise. Phosphocreatine (PCr) very rapidly replaces ATP during high intensity anaerobic exercise, as glycogen also rapidly replaces ATP, but it moderately rapidly replaces ATP during endurance aerobic exercise. In the end, fatty acids replace ATP during aerobic endurance exercise, but less rapidly (Williams, 2012).

Besides the intensity of exercise, other factors also affect which substrate will be utilized for energy production, including diet quality, lean body mass, fat body mass, estimated physical activity level, and sex (Gonzalez and Stevenson, 2012).

Also, duration of exercise has an impact on energy production in muscles. The ability of skeletal muscles to use dietary fats as a fuel increases as exercise duration increases, and can spare muscle glycogen supplies (Hunter, 2012).

Skeletal muscles are crucial for physical function, and are highly adaptable to environmental changes, such as diet and physical activity levels. More precise, skeletal muscles are highly adaptable to alterations in substrate availability and can switch between glucose and fat

oxidation (Jeromson et al., 2015). Important feature of healthy skeletal muscles is metabolic flexibility. It is the ability of muscle tissue to switch from one substrate for energy production to another when required.

Metabolic flexibility in human muscle cells includes suppressibility (the ability of glucose to suppress fatty acid oxidation), adaptability (the ability of cells to increase fatty acid oxidation when fatty acid availability increases) and substrate-regulated flexibility (the ability to increase fatty acid oxidation when changing from a high glucose, low fatty acid condition to a high fatty acid, low glucose condition) (Da Boit et al., 2017; Hessvik et al., 2010). In vitro studies have shown a favorable effect of omega-3 PUFAs administration on skeletal muscle metabolic flexibility (Hessvik et al., 2010).

Therefore, the type and amount of fat in the diet are important for the whole-body metabolic health and physical performance of an athlete (Jeromson et al., 2015).

2.3.1. Endurance exercise and fat metabolism

Endurance exercise, by actuating multiple body mechanisms, enhances the use of fat as energy source during aerobic exercise. Hence, endurance athletes are better fat burners (Williams, 2012).

Characteristic of endurance exercise are repeated isotonic contractions of large skeletal muscles. This type of aerobic exercise is ordinarily performed at submaximal intensity, with the main purpose of progressively moving the start of anaerobic metabolism and lactate production towards higher exercise intensity. Examples are running, swimming, cycling, cross-country skiing, and speed skating (Morici et al., 2016).

Skeletal muscle contraction is enabled by use of carbohydrates and fats as fuels during exercise. Fat is considered quantitatively the most important fuel for most endurance exercises (Jeukendrup and Aldred, 2004). Glycogen, especially muscle glycogen, is an important fuel at high exercise intensities. Depletion of glycogen stores can result in fatigue and may lead to reduction in exercise intensity or termination of exercising. Stores of glycogen in the body are quite small comparing to fat stores and can be depleted within approximately one hour of exercise. Conversely, much larger fat stores in the body would be sufficient for long lasting endurance exercises (Jeukendrup and Aldred, 2004).

Therefore, adaptations for sparing glycogen stores and increasing fat oxidation are desirable in endurance exercise training, and could lead to improvements in performance (Mickleborough, 2013; Jeukendrup and Aldred, 2004).

Aerobic lipolysis of fatty acids during muscle contractions in endurance exercise can significantly contribute to energy production in muscles. The source of these fatty acids can be both intramuscular triglycerides (i.e. fat energy stores in muscle cells) and free fatty acids delivered to muscles by blood from adipose tissue or liver (i.e. dietary fat from food ingestion) (Williams, 2012).

Optimizing the use of fat for energy production could be useful for endurance athletes to spare liver and muscle glycogen, through improvements in insulin sensitivity and glucose tolerance, for the later stages of an aerobic endurance exercise (Gonzalez and Stevenson, 2012; Williams, 2012).

Moreover, enhanced fat oxidation could be considered as a goal for both athletes and the general population, which can potentially improve performance for the former and health for the latter (Gonzalez and Stevenson, 2012).

2.3.2. The effect of omega-3 PUFAs on endurance exercise performance

According to previous findings, omega-3 PUFAs might be beneficial for endurance athletes who rely on fatty acids as substrate to sustain prolonged exercise (Da Boit et al., 2017).

For the past few decades, scientists have been studying whether increased intake of EPA and DHA could be beneficial for athletes in terms of enhancing aerobic energy supply (Peoples and McLennan, 2016). Recent scientific evidence suggests that changes in the omega-3 PUFAs content of skeletal muscle may influence both muscular and neuromuscular function and metabolism (Peoples and McLennan, 2016; Jeromson et al., 2015).

Essential omega-3 fatty acids, as a source of energy, are important in sports nutrition (Williams, 2012). As omega-3 fatty acids, primarily EPA and DHA, have shown the potential to improve general health and physiological functions, they have become a central research interest in nutrition science (Peoples and McLennan, 2016; Kawabata et al., 2014). Furthermore, other studies in humans demonstrated that omega-3 PUFAs can influence the exercise and nutritional response of skeletal muscles. These studies show that the prior

omega-3 status influences the metabolic response of muscle to nutrition, and also the functional response to a period of exercise training as a stimulus (Jeromson et al., 2015). Many studies have attempted to determine whether omega-3 fatty acid ingestion could improve various aspects of physiological performance. However, there is not much evidence of enhancement of physical performance after increased intake of either EPA or DHA (Williams, 2012; Peoples and McLennan, 2016). The required amount of omega-3 for athletes is not clearly defined, although consumption of 1-2 g of fish oil (EPA + DHA) per day was recommended as beneficial in training and competition (Drobnic et al., 2017; Peoples and McLennan, 2016; Micklebotough, 2013).

Increased intake of essential omega-3 fatty acids, EPA and DHA, have been theorized to improve endurance exercise efficiency in many ways, and there are several explanations for mechanisms by which it could be accomplished (Williams, 2012).

2.3.2.1. Skeletal muscle oxygen consumption

Possible mechanisms for improvements in muscle oxygen consumption include enhancements in endothelial and vascular functions and altered eicosanoid profiles (Rontoyanni et al., 2012).

Demand for oxygen is generally increased during endurance exercise (Peoples and McLennan, 2016).

EPA and DHA incorporate into cell membranes of various tissues, including red blood cells (erythrocytes), myocardial cells and skeletal muscle cells (Peoples and McLennan, 2016; Kawabata et al., 2014; Gonzalez and Stevenson, 2012). They alter membrane fatty acid composition and modify functions of the heart and skeletal muscles (Peoples et al., 2008). The increase of omega-3 PUFAs in erythrocyte membrane phospholipid composition minimizes the loss of its membrane fluidity and facilitates the transport of erythrocytes through the bloodstream (Jeukendrup and Aldred, 2004). Such effects would enhance blood flow and by that oxygen delivery to working skeletal muscles and contribute to the improvement in peripheral oxygen supply. Ultimately, it could result in improved exercise performance (Peoples and McLennan, 2016; Mickelborough, 2013; Jeukendrup and Aldred, 2004).

Above mentioned could also increase adipose tissue blood flow, and a result could be enhancement in lipid utilization which may be a limiting factor for fatty acid availability during exercise (Gonzalez and Stevenson, 2012). Increase in membrane composition of

omega-3 PUFAs in myocardium improves function of the heart, particularly in the event of compromised oxygen supply or declining contractility (Peoples and McLennan, 2016; Peoples et al., 2008).

Heart rate is associated to exercise intensity and is a strong predictor of myocardial oxygen consumption. Fish oil, by increasing DHA concentration in the myocardial membranes, causes a slowdown of the heart rate and improves efficiency of the heart. Thus, heart rate is lower following dietary provision of fish oil and oxygen saturation of tissues increases. Simply put, heart rate is lower but more blood, i.e. oxygen, is delivered in each heartbeat (Peoples and McLennan, 2016). DHA from fish oil can also incorporate into the mitochondrial membranes in skeletal muscle cells, which increases concentration of PUFAs in those. Similarly, as the enhancement in oxygen saturation in metabolically active heart, through enhanced mitochondrial function dietary fish oil may provide improved skeletal muscle function and fatigue resistance (Hingley et al., 2017; Peoples and McLennan, 2016). In other words, omega-3 fatty acids increase oxygen delivery to the heart muscle so that the heart does not have to work as hard to get the oxygen it needs for its functions (Simopoulos, 2007). Fish oils may act within the healthy heart and skeletal muscle to reduce both whole-body and myocardial O_2 demand (i.e. the amount of oxygen that the heart requires to maintain optimal function) during exercise, without a decrement in performance (Peoples et al., 2008). The reduced whole-body O_2 consumption observed after fish oil supplementation may reflect reduced (but more efficient) use of O_2 by active skeletal muscle, the largest consumer of oxygen during exercise (Peoples et al., 2008).

Another potential explanation for fish oil supporting physical performance could be the temporal inflammatory, immunomodulation and oxidative stress responses, through the production of various metabolites (e.g. eicosanoids), to exercise associated with prolonged or intense physical exhaustion (Peoples and McLennan, 2016). Oxidative stress increases after a bout of intense exercise, and fish oil is effective in the prevention and treatment of inflammatory conditions (Jeukendrup and Aldred, 2004). Fish oil supplementation may prevent muscle damage induced by an acute inflammatory response caused by a bout of intense exercise. Inflammation and inflammatory responses are maintained in the muscle tissue by local and systemic elevation of specific metabolites (Macaluso et al., 2013). Exercise may influence the production of eicosanoids, as increased eicosanoid production is observed immediately following exercise (Markworth et al, 2013).

It has been proposed that exercise-induced vasodilation might be via the action of locally produced metabolites (i.e. eicosanoids) whereby EPA and DHA attenuate vasoconstriction and enhance dilatation and blood flow to contracting skeletal muscles (Rontoyanni et al., 2012). Hence, by influencing the production of eicosanoids, fish oils could improve blood flow and blood pressure. Omega-3 polyunsaturated fatty acids (PUFAs) have been shown to decrease the production of inflammatory eicosanoids and other proinflammatory species, thus have anti-inflammatory and immunomodulatory response to exercise (Mickleborough, 2013).

2.3.2.2. Glucose utilization

In humans, omega-3 PUFA also has been shown to improve insulin sensitivity in skeletal muscles (Philpott et al., 2019). Increased omega-3 PUFA levels in skeletal muscle membranes enhances cell membrane fluidity. Hence, the number of insulin receptors on it increases and cell insulin affinity enhances, thereby increasing the amount of glucose that could be utilised by muscle (Philpott et al., 2019). An increase in insulin sensitivity leads to greater muscle glycogen resynthesis and the subsequent potential to increase carbohydrate oxidation rates and decrease fat oxidation rates. During endurance exercise, a shift in substrate utilization from fat to carbohydrate would reduce the volume of oxygen used for ATP resynthesis, and in turn improve the exercise efficiency (Philpott et al., 2019). This could improve muscle function and change substrate preference, which lead to resistance to muscle fatigue and sustained steady state of contraction during endurance exercise (Peoples and McLennan, 2016; Peoples et al., 2008).

Thus, by affecting expression of specific genes connected to glucose and lipid metabolism, omega-3 PUFAs allow a greater reliance on fat for fuel during endurance exercise, while sparing muscle glycogen and improving body composition and possibly exercise performance (Mickleborough, 2013).

2.3.2.3. Calcium handling

Dietary fish oil has been shown to improve calcium ions handling in the heart and skeletal muscles and reduce O_2 consumption attributed to excessive sarcoplasmic reticulum calcium

cycling. The sarcoplasmic reticulum membrane also highly incorporates DHA from dietary fish oil. Improvement in calcium handling by skeletal muscle sarcoplasmic reticulum could be a factor contributing to reduced O_2 demand of contracting skeletal muscle as in the heart (Peoples et al., 2008). In the myocardium omega-3 PUFAs act directly to improve calcium handling and in skeletal muscle reduce fatigue-related decline in maximum rates of contraction and relaxation (Peoples and McLennan, 2016). So, when amount of omega-3 PUFAs, especially DHA, in the membrane increases the process of intracellular Ca^{2+} pumping is optimized, supporting force production (Hingley et al., 2017).

Also, there is a possible link between calcium intake and substrate utilization. Intracellular calcium has been associated with insulin resistance in obesity, but is also thought to be a key regulator in adipocyte lipolysis and lipogenesis. A lot of theories support the role of Ca intake in both muscle and adipocyte fat metabolism (Gonzalez and Stevenson, 2012).

2.3.3. Omega-3 PUFAs dietary supplements

Generally, the optimal diet for human health is also optimal for performance for most athletes, and it includes both whole foods and food supplements (Peoples and McLennan, 2016; Williams, 2012).

Dietary supplements are often used as part of personal health care to ensure adequate intake of nutrients, maintain health, and prevent or help in treatment of various health problems (Swanson et al., 2012). Supplements are often promoted as providing potential health benefits beyond basic nutrition, especially for groups like physically active people and athletes. That is one of the reasons for high use of dietary supplements by athletes which has been observed worldwide, and nowadays it became a common part of training and competition (Peoples and McLennan, 2016; Swanson et al., 2012; Williams, 2012).

Interest in potential benefits of omega-3 PUFA supplementation in athletes has been increasing, with the assumption it can improve athletic performance (Da Boit et al., 2017). This assumption was based on the well-known variety of beneficial effects which EPA and DHA have on general health (Drobnic et al., 2017).

Fish oil is naturally high in very long-chain omega-3 PUFAs, EPA and DHA, which makes it a good dietary source for supplementation (Shahidi and Ambigaipalan, 2018; Vannice and Rasmussen, 2014). It could be obtained from oily fish flesh or lean fish livers (Calder, 2012).

Fish oil has greater health benefits than plant sources of omega-3 PUFAs because of its higher concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Walker et al., 2015).

Application of fish oil into different products (e.g. dietary supplements, fortified foods and beverages, infant formulas, and pharmaceuticals) is rapidly increasing nowadays, as well as their consumption (Hernandez, 2016; Kritchevsky, 2008). Although, the incorporation of omega-3 PUFAs into different foods and beverages is often challenging due to their low water-solubility, poor oxidative stability, and variable bioavailability. The concealing of the intense fish flavor can also be a challenge (Walker et al., 2015).

Given that the consumption of dietary supplements is a growing trend, experts in the field of food technology are constantly trying to find a way to improve their properties and quality.

The absorption level of omega-3 PUFAs from fish oil supplements is variable, due to its poor bioavailability. Type of the omega-3 supplement, and form of the fish oils inside it, highly affects how well they can be absorbed by the human body. One of the factors that limits the efficiency of fatty acid uptake during digestion is the emulsification.

Several studies show that pre-emulsification of an oil mixture increases the digestion and improves the absorption of the omega-3 PUFAs, especially EPA and DHA, in comparison to standard non-emulsified products (Garaiova et al., 2007).

Pre-emulsification process is an excellent way of improving the availability of omega-3 PUFAs in the body, making the uptake of the latter much easier and faster, which can be especially useful for athletes (Walker et al., 2015; Garaiova et al., 2007).

3. EXPERIMENTAL PART

3.1. EXPERIMENTAL DESIGN

This laboratory-controlled study was double-blinded, randomised, and cross-over in design. Ethical consent for this study was approved by the NHS, Invasive & Clinical Research (NICR) ethics committee at the University of Stirling. All testing was performed in accordance with the standards and principles set by the Declaration of Helsinki.

Participants attended the Sport and Exercise Physiology Laboratory at University of Stirling, Stirling, United Kingdom, on five separate occasions, with each testing day separated by a period of at least one week. Testing took place during a participants' noncompetitive season to minimize the influence of training and dietary supplementation on performance. All testing was conducted in the morning, because the participants were asked to arrive to the laboratory in a fasted state. There is scientific evidence which indicates positive effects of the long chain n-3 PUFAs when assessed after an overnight fast (Armah et al., 2008). Participants were also asked to abstain from alcohol and vigorous exercise for 24 hours before each visit to the laboratory. Food diaries were recorded for three days prior to each laboratory visit and were replicated before each subsequent visit. This dietary control was administered to prevent the potential confounding effect (in addition to supplementation) on substrate utilization and performance. Participants were also asked to wear the same clothing during each testing session, because it could affect their comfort, thermoregulation and consequently performance (Gavin, 2003; Zhang et al., 2002).

On the initial visit to the laboratory (Visit 1), participants were given a verbal explanation of the study protocol. Next, participants signed the consent forms, and completed the necessary pre-participation health questionnaire and a food frequency questionnaire (FFQ) based on their dietary habits specific to omega-3 PUFA consumption. Subsequently, participants' fasted body mass, height and seated blood pressure were measured. Thereafter, a blood sample was taken for determination of participants' baseline omega-3 index. Finally, participants conducted preliminary testing (pre-testing) to determine their eligibility for the study, by completing lactate threshold and maximum oxygen uptake (VO_{2max}) tests.

All participants who met the eligibility criteria were asked to visit Sport and Exercise Physiology Laboratory on four additional occasions. The initial visit was followed by a

familiarisation trial (Visit 2) in order for the participant to gain experience of what is required in the testing trials. No supplement was consumed during this visit. An additional purpose of the familiarisation was to diminish the learning effect on results during testing trials.

Each participant undertook three additional study visits to the laboratory (Visit 3, 4 and 5) that were separated by a one-week wash-out period. Participants visited the exercise laboratory weekly at the same time in the morning. Trials consisted of resting for 60 minutes, two hours of pre-loaded submaximal cycling and then performing a work target time trial. The time trial testing protocol has been shown to be valid and reliable in investigating differences in performance between use of test supplements (Currell et al., 2006; Jeukendrup et al., 1996; Hickey et al., 1992).

A schematic of the experimental protocol is shown in Figure 6.

All participants received all three supplement conditions and the order of supplement administration was randomly assigned after confirmation of their eligibility for this study. Every participant acted as their own control, as this was a cross-over study. Randomisation was performed by a member of research group and staff of Sport and Exercise Physiology Laboratory at University of Stirling, but who was independent of this study.

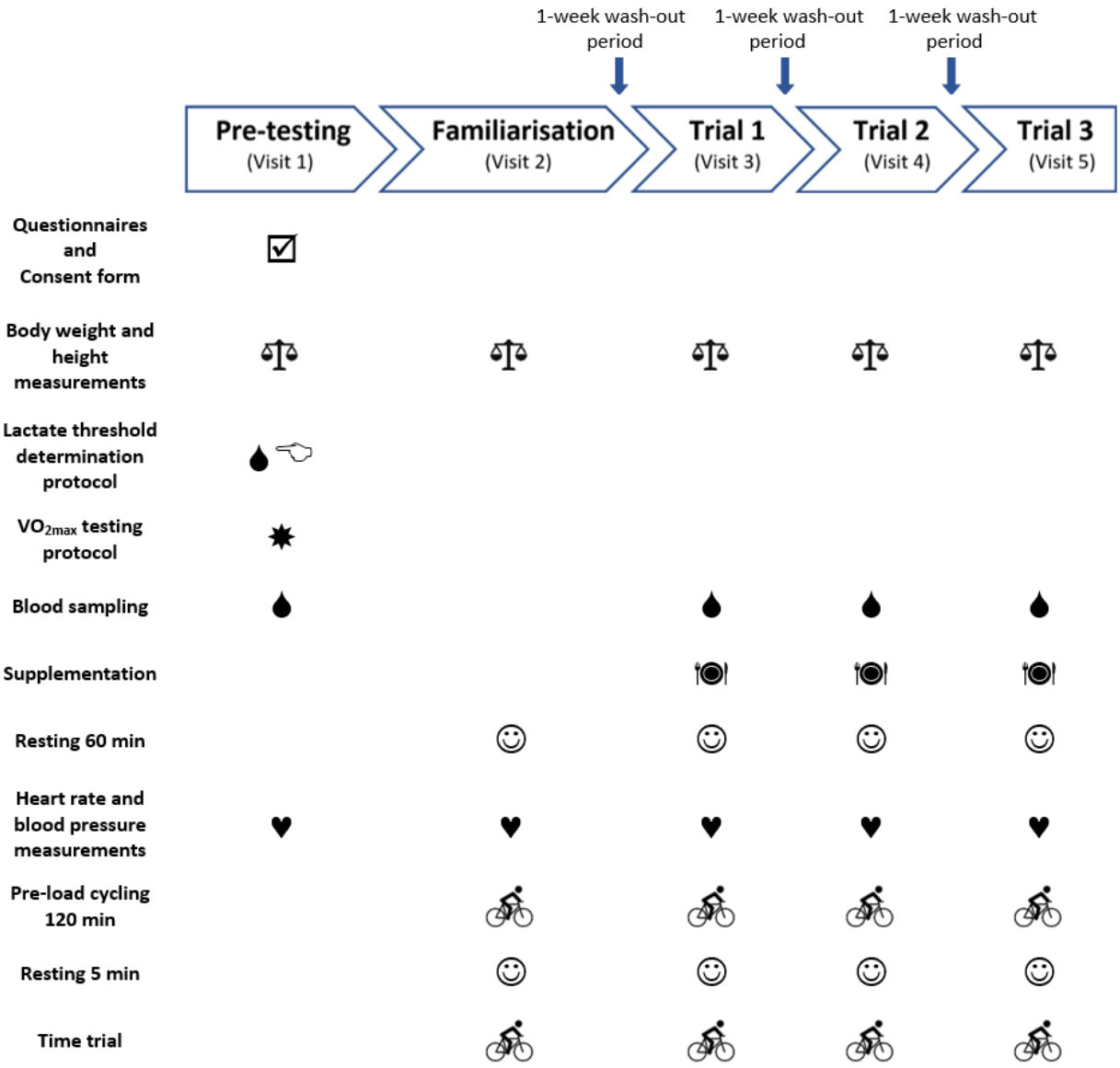


Figure 6. Schematic of experimental study design

3.2. PARTICIPANTS

The target population was well trained healthy male cyclists, who do not consume a diet high in omega-3 polyunsaturated fatty acids. Cycling was selected as the endurance exercise activity because it is a continuous and steady effort sustained for an extended period of time and the specificity of the cycle ergometer to the trained cyclists. Only male participants were

recruited, so that the effects of the menstrual cycle and/or oral contraceptives could be avoided.

The principal inclusion criteria for participation in this study were: male sex, well trained cyclists, age 18 to 35 years, healthy and low baseline omega-3 index. Also, it has been required from participants to have been cycling at least twice per week during the previous six months and to have a VO_{2max} of at least 55 $ml \cdot kg^{-1} \cdot min^{-1}$, which ensured that experienced cyclists were recruited and that they could complete the testing protocol.

The exclusion criteria used to eliminate factors which might have influence were regularly high consumption of a fatty fish and fish oil or use of its supplements, smoking, taking part in other studies, and any health disorder identified as a risk factor.

Participants were screened during their initial visit to the laboratory, when they filled in a pre-participation health questionnaire and food frequency questionnaire based on their omega-3 intake. Moreover, a blood sample was taken to determine their baseline omega-3 index.

The purpose, procedures and risks associated with participating in this study were explained in detail to each participant before they decided to volunteer and provided the written and verbal informed consent to participate in this study. Eligible participants attended one pre-screening session, one familiarisation session and three exercise trials.

The recruitment process yielded seven participants (N = 7) who met the eligibility criteria. Their main characteristics are shown in Table 1. Participants were recruited from cycling clubs at the University of Stirling and surrounding area through the use of posters, flyers and advertisements. Each participant served as their own control.

Table 1. Main characteristics of study participants (N = 7)

Participant characteristics	**Mean ± SD**
Age [years]	25,3 ± 3,86
Body weight [kg]	79,5 ± 9,21
Body height [cm]	183,1 ± 5,56
BMI [kg/m^2]	23,71 ± 2,58
VO_2 max [mL/kg/min]	61,98 ± 5,63

Data presented as Mean ± SD.

BMI = body mass index, VO2max = maximal oxygen uptake

3.2.1. The Omega-3 Index

The omega-3 index is a measure of omega-3 polyunsaturated fatty acids incorporated into red blood cells, i.e. erythrocytes (Allaire et al., 2017). Omega-3 index reflects the omega-3 fatty acid status of major tissues, and therefore represents an individual's general status in eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), and is used as a biomarker of omega-3 fatty acid status in the whole organism (Allaire et al., 2017; Drobnic et al., 2017; Harris and Polreis, 2016; von Schacky et al., 2014).

This figure is calculated by comparing the sum of incorporated EPA and DHA to total erythrocyte fatty acids present, and it is expressed as a percentage (Allaire et al., 2017; Drobnic et al., 2017; von Schacky, 2014). A value under 5% is considered as low omega-3 index and has been associated with a higher risk of detrimental cardiovascular and neurological events. Conversely, an omega-3 index of 8-11% has been recommended as the optimal range for health, and has also been considered to be adequate in elite athletes (Drobnic et al., 2017; von Schacky et al., 2014). Studies have confirmed that adequate intake of EPA and DHA is associated with higher values of the omega-3 index (Drobnic et al., 2017), which indicates that health, and consequently the performance, may be improved by raising the levels of omega-3 PUFAs in athletes with a low omega-3 index.

3.3. MATERIALS AND METHODS

3.3.1. Materials

3.3.1.1. Devices for anthropometric measurements

Body weight and height were measured at the beginning of each laboratory visit. These measurements were conducted to ensure no significant variations in body weight and BMI between visits. Participants performed both measurements in a fasted state, barefoot and wearing minimal clothing. Height was measured using a stadiometer (model Harpenden Stadiometer, Holtain Ltd., United Kingdom), and weight was measured using a scale (model Seca QUADRA 808, Germany). Body mass index (BMI) was calculated as participant's weight in kilograms divided by the square of his height in meters.

3.3.1.2. Equipment for haemodynamic measurements

To obtain blood samples, a cannula was inserted into the participant's antecubital vein and all the blood sampling was done by trained phlebotomists. Blood samples were collected into vacutainer tubes which contained an anti-coagulant (K2EDTA) to prevent clotting of the blood. At each timepoint, 6 mL of blood was dispended and were stored on ice immediately after collection. After every trial, blood samples were centrifuged, plasma samples were separated in Eppendorf tubes and stored into a freezer at temperature of −80 ^{o}C until those were further analysed.

During the preliminary fitness testing, capillary blood was drawn from a pricked fingertips for analysing the lactate concentration in the blood. Blood sample was collected on the strip and inserted into the lactate analyser (LactatePro LT–1710, ArkRay Inc., Kyoto, Japan) to determine blood lactate level.

Heart rate was monitored using a standard chest strap monitor, sport watch and a heart rate sensor (Polar Electro, Finland) worn by participants during all cycling protocols.

Systolic and diastolic blood pressure was measured manually, using a manual sphygmomanometer (model Accoson greenlight 300, United Kingdom) and a stethoscope (model Littmann classic 2, United States of America). Blood pressure measurements were taken both when the participant was relaxed in a seated position, and during the 120 min of pre-load cycling exercise. All measurements of blood pressure were taken on the participant's left arm and by the same person to reduce measurement error. Before measuring during exercise, participants relaxed the arm in a vertical plane next to their body.

3.3.1.3. Cycle ergometers

All testing protocols that included cycling exercise were completed on an electronically braked Lode cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Seat height and handlebar reach were optimized for each participant.

3.3.1.4. Equipment for measuring maximum oxygen uptake (VO_{2max})

The maximum rate of oxygen uptake (VO_{2max}) was measured during cycling exercise.

Participants wore a chest strap heart rate monitor and a face mask which was connected by a gas flow tube to the rapid-response gas analyzer. Oxygen taken in and used by the participants was measured with an automated online gas analysis machine (Oxycon Pro, Jaeger, Germany). This machine was also connected to a computer, estimating VO_{2max} as the highest average value over a 30 second period.

3.3.1.5. Test supplements

Each participant consumed each of the three test supplements once, and the order of that was randomly assigned. The test supplement treatments were emulsified omega-3 (supplement A), placebo (supplement B) and non-emulsified omega-3 (fish oil) supplement (supplement C).

The supplements weighed 85 grams and they were packed in sachets. They were matched for appearance and taste to ensure blinding. Also, all the supplements were nutritionally matched, meaning each contained 21 grams of carbohydrates and 8.3 g of fat.

The emulsified omega-3 supplement contained 5 g of EPA and 3.3 g of DHA. The placebo was emulsion of palm oil and soybean oil in a 4:1 ratio, and the use of placebo of that composition is consistent with previous placebo-controlled research (Minihane et al., 2015; Chong et al., 2010; Hamazaki et al., 1996). The fish oil supplement contained the same amount of EPA (5 g) and DHA (3.3 g) as emulsified omega-3 supplement, but only difference was that this supplement was not emulsified.

Supplements were provided in a double-blind manner, which means that neither the investigators or participants knew which of the test supplements they are consuming.

3.3.2. Methods

3.3.2.1. Preliminary testing

On the initial laboratory visit, participants signed the consent forms, completed FFQs, their anthropometric measurements in fasted state were taken and finger prick blood sampling was conducted for determination of baseline omega-3 index. Next, participants undertook preliminary testing on the cycle ergometer. There were two baseline aerobic fitness tests to determine the participants eligibility for the study, a lactate threshold test followed by

maximum oxygen uptake (VO_{2max}) test. Participants were instructed to do a 10 minutes of a light self-selected cycling intensity as warm-up before the first test began, and to become familiar with the equipment.

The lactate threshold could be described as the maximal level of exercise intensity that can be maintained over a prolonged period of time with little or no increase in lactate concentration in the blood (UVA, 2020). More precisely, it is the point at which lactate accumulates in the blood stream at a higher rate than it can be removed from it, what hinders muscles' ability to contract and causes fatigue.

The lactate threshold test is an incremental exercise protocol. The test began with the initial resistance of 120 Watts, and exercise intensity increased by 20 Watts every 3 minutes. Blood samples were taken from the participants' fingertips at the end of each interval for the assessment of blood lactate concentrations. In other words, the level of blood lactate was recorded at the end of each 3 minutes interval. The exercise intensity was continued to increase until the blood lactate concentration was at least 1 mmol/L higher in the end of two consecutive intervals, and that point was considered the lactate threshold (UVA, 2020; Newell et al., 2015; Aunola and Rusko, 1984).

To measure blood lactate concentrations, capillary blood drawn from fingertips was collected on the strips and inserted into the lactate analyser which recorded blood lactate level. The testing protocol was stopped when blood lactate increased by 2 mmol/L from the previous stage and lactate threshold was determined as the exercise intensity (i.e. wattage) reached on the penultimate stage of the test. This test was followed by a ten-minute rest before the next test was conducted.

The maximum oxygen uptake (VO_{2max}) test is an incremental test used for direct measurement of the maximum (peak) amount of oxygen a person can utilize during intensive exercise such as cycling. It is a common for evaluation of the cardiorespiratory fitness, to establish the maximal aerobic capacity of an athlete (Ekblom-Bak et al., 2012; Hunter, 2012). Unit of measure for VO_{2max} is mL of O_2 per kg body weight per minute. Starting exercise intensity on the cycle ergometer for this purpose was the penultimate wattage each participant achieved during the lactate threshold testing. The exercise intensity continued to increase by 30 Watts every minute until participant's voluntary exhaustion. During the whole procedure, participants were wearing a face mask connected to a calibrated machine for gas analysis for continuous measuring of respiratory gas exhalation, which was connected to a computer. The

VO_{2max} was automatically calculated using the highest average values over a 30 second periods.

The VO_{2max} test end time and power output of the stage were used to calculate peak power output (PPO). The maximum workload or peak power output is defined as the athlete's maximum work rate that could be sustained for a very short time during progressive incremental exercise to exhaustion. It is also a predictor of endurance performance (Hawley and Noakes, 1992) and has been shown to be highly related to time trial (TT) performance velocity (Bentley et al., 2001).

Peak power output (PPO) was calculated using the equation:

$$\text{PPO} = \text{final wattage} + ([\text{time} / 60 \text{ seconds}] \cdot \text{PI}) \quad [1]$$

where W_{final} is exercise intensity expressed in Watts reached on the final stage of VO_{2max} testing, time refers to the time in seconds spent in the last stage of VO_{2max} testing, 60 seconds is the duration of each stage of VO_{2max} testing, and PI is exercise intensity increase of 30 Watts per stage of VO_{2max} testing.

The results collected during the preliminary fitness test were used as a basis for the testing trials. In this regard, the resistance required for each participant in cycling testing trials was calculated based on those results (i.e. participants cycled at a set percentage of VO_{2max} during the testing trials).

3.3.2.2. Familiarisation and testing trials

A familiarisation of the testing protocol was the same as the testing trials, except participants have not consumed any supplement and no haemodynamic parameters were measured.

Each testing trial consisted of pre-load submaximal cycling and time trial.

Participants were instructed to arrive at the laboratory on each testing day in a fasted but euhydrated state, meaning that participants were not allowed to consume any food or drink other than water from 22:00 hours the night before the trial. Nothing but one of the three test supplements participants consumed until the end of each trial.

Environmental room conditions in the laboratory were controlled at 18 - 21 °C and 45 % - 55 % relative humidity.

Upon participant's arrival to the laboratory, body mass and height were measured, a cannula was inserted in the antecubital vein to allow blood sampling, and resting measurements of blood pressure and a blood sample were taken. Then, one of three test supplements were consumed and 60 minutes of rest for supplement to start digesting followed. Each participant received all treatments on the same day of the week and had one-week washout period between treatments. A washout period serves to minimize the possibility of the preceding treatment to influence on the response to the next treatment (i.e., to prevent carryover or drug residual effects of treatments), and it is most commonly applied between several consecutive testing trials.

One hour after ingestion of the supplement, participants cycled for 120 minutes at 95% of their lactate threshold. During that pre-load cycling, heart rate was recorded in intervals of 15 minutes and blood pressure and venous blood samples were taken every 30 minutes. Participants were permitted to drink water during pre-load cycling sessions, but not during the time trial. When that was finished, participants had 5 minutes to rest, use a toilet and it was needed to set up the equipment before the time trial. Then, participants were supposed to complete a time trial.

The target workload (in kilo Joules, kJ) was set so the participants were expected to complete the time trial as fast as possible. During the time trial distractions were kept to a minimum to avoid any possible external effect on the performance. The only feedback participants received was the amount of work that was left to be completed. It was displayed on a screen in front of the cycle ergometer. The targeted amount of work was specifically calculated for each participant using following formula:

$$\text{Work target} = (0.7 \times \text{PPO}) \times 1800 \quad [2]$$

where Work target is the targeted amount of work (in kilo Joules, kJ), PPO is peak power output (in Watts) calculated during the preliminary fitness tests and 1800 is the estimated time to completion in seconds.

It was also needed to calculate the alpha-value for each participant to determine the resistance on the cycle ergometer for the time trial. Equation used for that purpose was:

$$\alpha = 70\% \text{ of PPO} / (\text{preferred cadence})^2 . \quad [3]$$

Cadence is the rate at which a cyclist is turning the pedals, thus it is the number of pedal revolutions per minute (RPM). The alpha-value was entered in cycle ergometer before each time trial. The participants were expected to complete the time trial as fast as possible but in

maximum of 45 minutes if they were to cycle at 70% of PPO the whole time. The time to complete the time trial protocol was recorded on the computer and handwritten by the investigator.

A final venous blood sample and blood pressure were measured within 5 minutes after completing a time trial.

3.3.2.4. Blood samples analysis

All venous blood samples were collected into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and stored on ice. The samples were centrifuged at 4°C, plasma was separated in Eppendorf tubes and stored in the freezer at –80 °C. The frozen plasma samples were later analysed at the Institute of Aquaculture at the University of Stirling by gas-liquid chromatography methods to determine their omega-3 PUFA levels.

3.3.2.5. Statistical analysis

Statistical analysis of the data was performed using SPSS Statistics for Windows (IBM® SPSS® Statistics Version 25.0, Released in 2017, Armonk, New York, United States), GraphPad Prism for Windows (GraphPad Software LLC, version 8.4.3., San Diego, California, United States) and Microsoft Excel 365 (Microsoft Office Excel 365, 2019).

Normality of distribution was assessed using the Shapiro-Wilk test and Kolmogorov-Smirnov test. Mean changes from baseline to post-time trial were calculated and compared between groups using Paired Samples t-Test. All statistical tests were performed at a significance level of 0.05, meaning that p value lower than 0.05 was considered significant. Data are reported as the mean and standard deviation (Mean ± SD).

4. RESULTS AND DISCUSSION

4.1. PURPOSE OF THE STUDY

This study was undertaken to investigate to what extent are essential omega-3 polyunsaturated fatty acids, EPA and DHA, able to influence physiological processes in the human body during endurance exercise and immediately after their intake. The primary aim was to test the effects of acute omega-3 supplementation on endurance exercise performance and efficiency in trained cyclists with a low omega-3 index.

It was hypothesized that acute ingestion of single dose of omega-3 PUFA will positively affect the substrate utilization and indirect markers of exercise efficiency (i.e. heart rate, blood pressure), and also improve performance in a work target time trial.

There is limited literature regarding the impact of single-dose of omega-3 PUFA on endurance exercise performance. Many previous studies which investigated its impact on exercise performance mostly have included a certain period of supplementation before testing, ranging from days to months. (Drobnic et al., 2017; Zebrowska et al., 2015; Kawabata et al., 2014; Peoples et al., 2008; Bortolotti et al., 2007) It is thought that some effects observed after long term consumption of omega-3 PUFAs may also be observed already several hours after ingestion of an single dose of omega-3 PUFAs, but possibly in lower intensity.

4.2. OMEGA-3 PUFA CONTENT OF WHOLE BLOOD

Seven male participants completed all trials in the present study. However, due to constraints caused by a global pandemic of coronavirus (COVID-19) analysis of the blood samples was done only for three participants (N = 3). Blood samples were taken while participants were resting before pre-load cycling and ingestion of the supplement (Pre), then at 0, 30, 60, 90, 120 minutes of pre-load exercise and post-time trial.

No significant differences in omega-3 PUFA to Total PUFA ratio in blood plasma were observed between non-emulsified omega-3 supplement and placebo, but a significant time × intervention ($p < 0.05$) effect was observed between emulsified omega-3 supplement and

placebo. Also, significant time × intervention effect ($p < 0.05$) was observed between emulsified and non-emulsified omega-3 supplements (Figure 7).

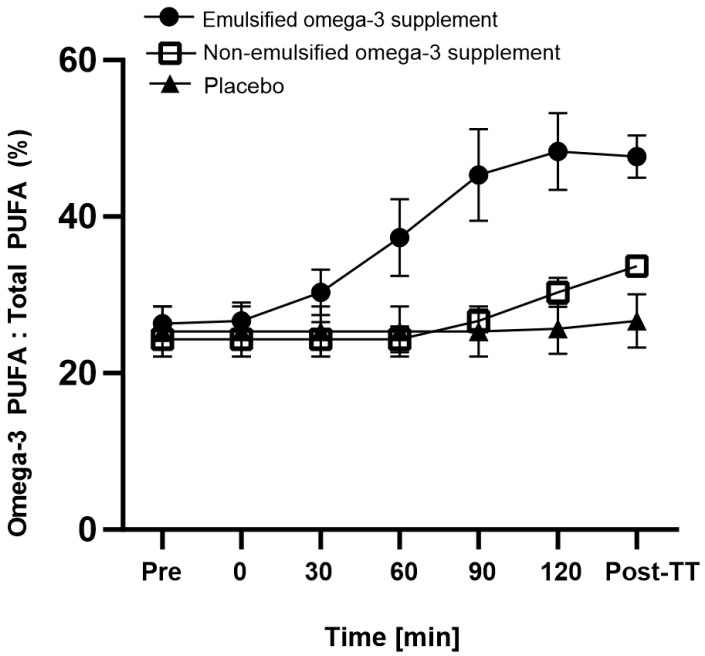


Figure 7. Mean omega-3 PUFA to Total PUFA ratio in blood plasma between trials

The omega-3 PUFA content in blood plasma appeared to reach the plateau in the period between the end of pre-load and the beginning of time trial, which is similar to previously reported results (Garaiova et al., 2007).

Results of blood analysis for EPA (Figure 8) and DHA (Figure 9) plasma levels have shown a significant time × intervention effect ($p < 0.05$) between trials.

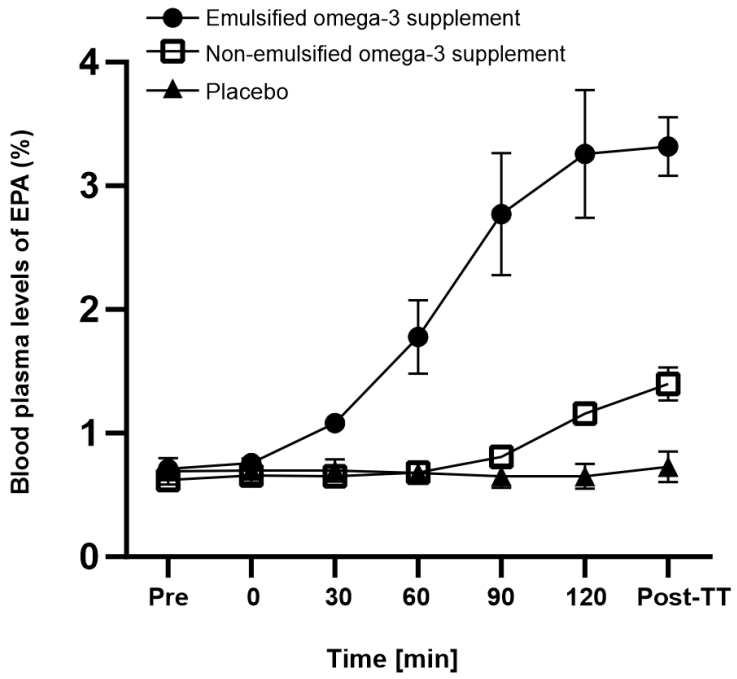


Figure 8. Mean levels of EPA in the blood plasma between trials

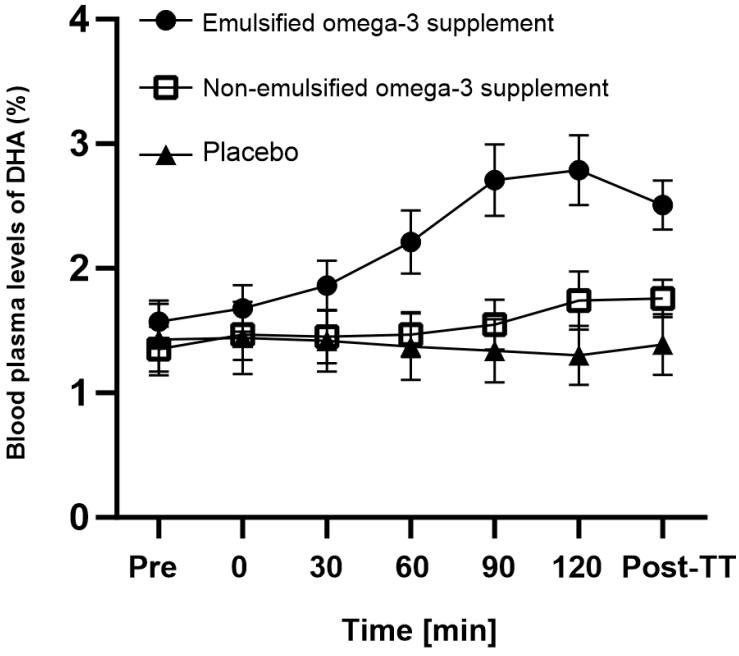


Figure 9. Mean levels of DHA in the blood plasma between trials

It can be concluded that there are differences between (all) test supplements for both EPA and DHA.

As EPA and DHA are constituent part of membranes of many cells, altering their level in the blood could affect heart and skeletal muscle functions (Peoples and McLennan, 2016; Kawabata et al., 2014; Gonzalez and Stevenson, 2012; Peoples et al., 2008). Increment of omega-3 PUFAs in the blood after their dietary intake, especially elevated DHA concentrations, lowers the heart rate and enhances efficiency of the heart (i.e. blood flow and oxygen delivery to skeletal muscles) (Hingley et al., 2017; Peoples and McLennan, 2016; Mickelborough, 2013; Jeukendrup and Aldred, 2004), what could result in overall improvement of exercise efficiency. Considering that one of the criteria for participation in this study was low omega-3 index, it would be advisable to increase the intake of omega-3 PUFAs in the everyday diet of these participants.

4.3. HEART RATE

Average values of heart rate measured during the pre-load exercise following consumption of each test supplements were 136 ± 15 for emulsified omega-3, 136 ± 14 for non-emulsified omega-3 and 133 ± 11 for placebo supplement. Measurements were taken at 15, 30, 45, 60, 75, 90, 105 and 120 minutes of pre-load exercise (Figure 10). Heart rate is expressed in beats per minute (BPM).

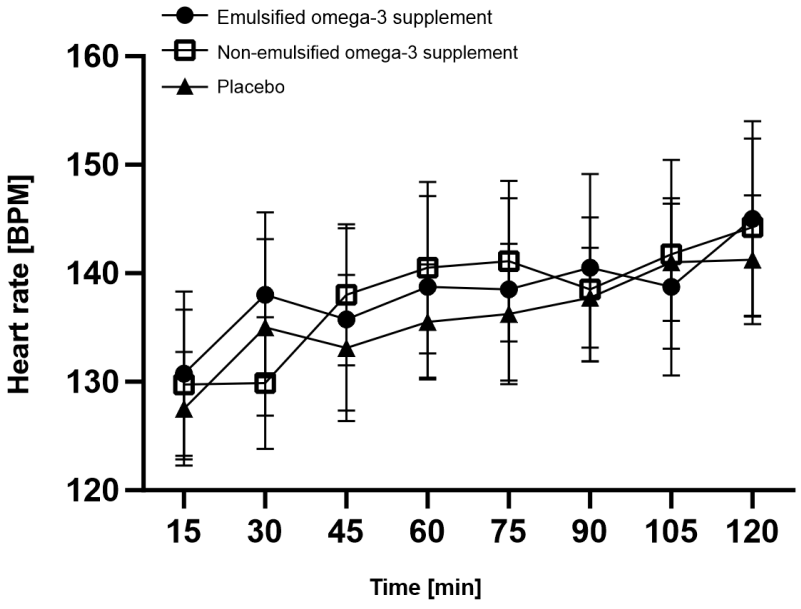


Figure 10. Heart rates over the 120 min submaximal pre-load cycling

One of the hypotheses was that heart rate would decrease during steady state exercise following a single-dose of an omega-3 supplement. Despite the increase in blood plasma concentration of omega-3 PUFAs, no differences in heart rate between trials were found. A study conducted previously (Rontoyanni et al., 2012) also found no differences in heart rate during exercise after supplementing with a single meal rich in either EPA or DHA compared to control. Obtained results are not in accordance with studies reported that omega-3 PUFA supplementation could reduce heart rate and oxygen consumption during submaximal exercise (Williams, 2012; Buckley et al., 2009; Ninio et al., 2008; Peoples et al., 2008). These data suggest that a single dose of omega-3 PUFA ingested shortly before exercise trial is not sufficient for this purpose, meaning that longer-term supplementation is needed to carry out beneficial effects on heart rate. Furthermore, measurement sensitivity could be questionable, because no measurements of heart rate were taken during the time trial in order not to distract participants. Aforementioned omega-3 PUFAs beneficial effects on cardiovascular function did not translate into an improvement in endurance performance.

4.4. BLOOD PRESSURE

Measurements of blood pressure taken before ingestion of test supplement (Pre) were considered as baseline. Baseline systolic blood pressure and was not significantly different ($p > 0.05$) between trials, nor was the values of baseline diastolic blood pressure (Figure 11).

Mean baseline systolic blood pressure was 121 ± 5 mmHg for placebo, 119 ± 5 mmHg for non-emulsified omega-3 and 116 ± 8 for emulsified omega-3 test supplement.

Mean baseline values of diastolic blood pressure were 80 ± 5 mmHg for placebo, 77 ± 9 mmHg for non-emulsified omega-3 and 79 ± 8 for emulsified omega-3 test supplement.

There were no time, intervention or time × intervention ($p > 0.05$) effects on systolic blood pressure throughout the pre-load exercise protocol (Figure 11). Likewise, no significant effects of time, intervention or time x intervention ($p > 0.05$) on diastolic blood pressure were found during the pre-load exercise (Figure 12).

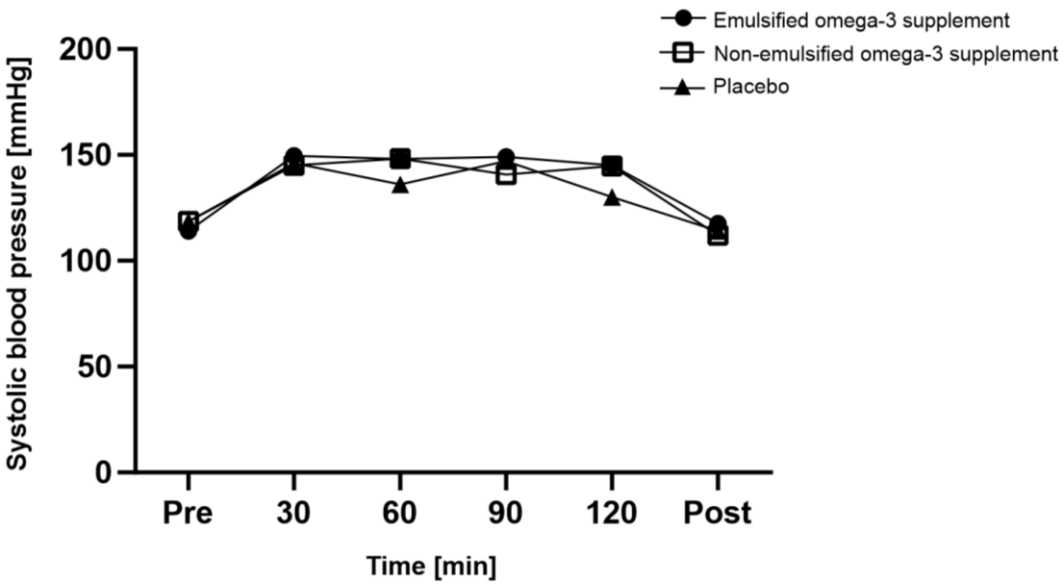


Figure 11. Systolic blood pressure measured before and during the 120 min submaximal pre-load exercise and post work-load time trial

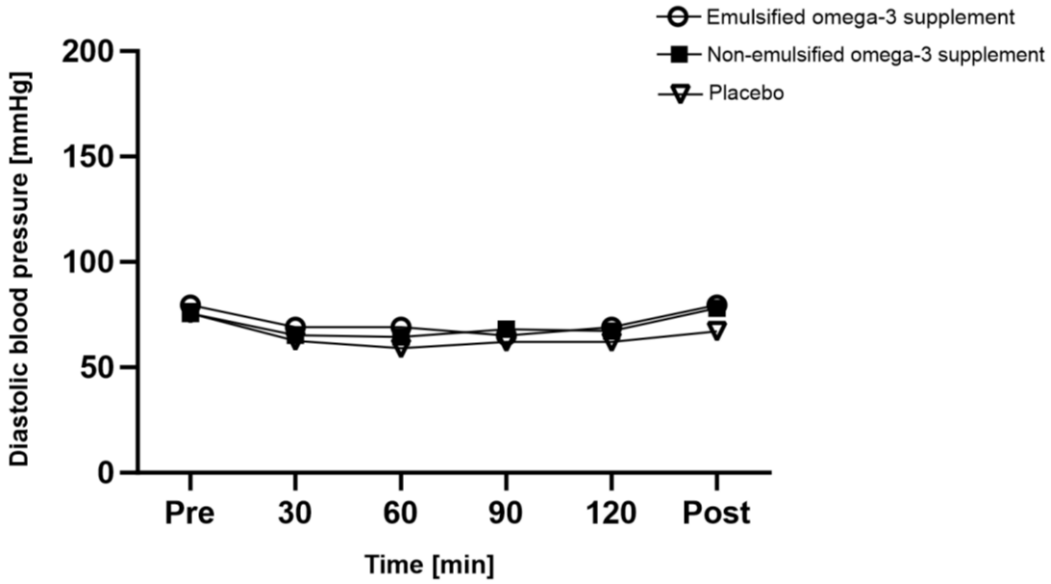


Figure 12. Diastolic blood pressure measured before and during the 120 min submaximal pre-load exercise and post work-load time trial

Post-time trial measurements of systolic and diastolic blood pressure demonstrated no significant differences between trials. Mean post-TT systolic blood pressure was 122 ± 10

mmHg for placebo, 113 ± 12 mmHg for non-emulsified omega-3 and 118 ± 3 for emulsified omega-3 test supplement.

Mean post-TT diastolic blood pressure was 77 ± 11 mmHg for placebo, 78 ± 3 mmHg for non-emulsified omega-3 and 80 ± 4 for emulsified omega-3 test supplement.

Exercise causes many physiological changes in the athlete's body, such as in blood pressure and blood flow. Another hypothesis in this study was that ingestion of a single-dose omega-3 supplement would decrease blood pressure during steady state exercise. Physical exertion represents one type of stress for a human organism, which answers in production of different eicosanoids, causing vasoconstriction. Precursors for the synthesis of these are omega-6 PUFAs, which are widely present in modern human diet and therefore in the human body. Short-term supplementation with omega-3 PUFAs may impact endurance performance by altering the omega-6 to omega-3 ratio. Omega-3 PUFAs have anti-inflammatory and immunomodulatory response to exercise (Mickleborough, 2013). By inhibition of omega-6 eicosanoid production, EPA and DHA attenuate vasoconstriction which results in vasodilation and enhanced and blood flow to contracting skeletal muscles, and also blood pressure. (Rontoyanni et al., 2012).

Contrary to some previously reported findings (Peoples et al. 2008; Buckley et al. 2009), but similar to results of other studies (Da Boit et al., 2017; Rontoyanni et al., 2012), no significant differences were observed in blood pressure between interventions. That indicates that acute supplementation with omega-3 PUFA did not improve cardiovascular function during submaximal endurance exercise. Also, participants were well-trained individuals, so it is possible that a single dose of omega-3 PUFAs cannot further amplify vasodilation. Also, in this study neither blood pressure was measured during the time trial in order not to distract participants so its impact at higher exercise intensities is not recorded.

In addition to blood flow, endurance performance is dependent on many other physiological factors, all of which interact during exercise. Hence, improvement in one physiological factor might be insufficient to improve the cycling performance overall.

4.5. WORK-TARGET TIME TRIAL

As mentioned previously, time trial protocols are valid for assessment of cycling performance. Thus, primary outcome measure in this study was the time to complete a set workload (i.e. time trial).

The main hypothesis was that endurance performance during time trial would improve with consuming a single-dose of omega-3 supplement just before the exercise.

Mean time for completion of the time trial was not significantly different ($p > 0.05$) when different test supplements were consumed (Figure 13).

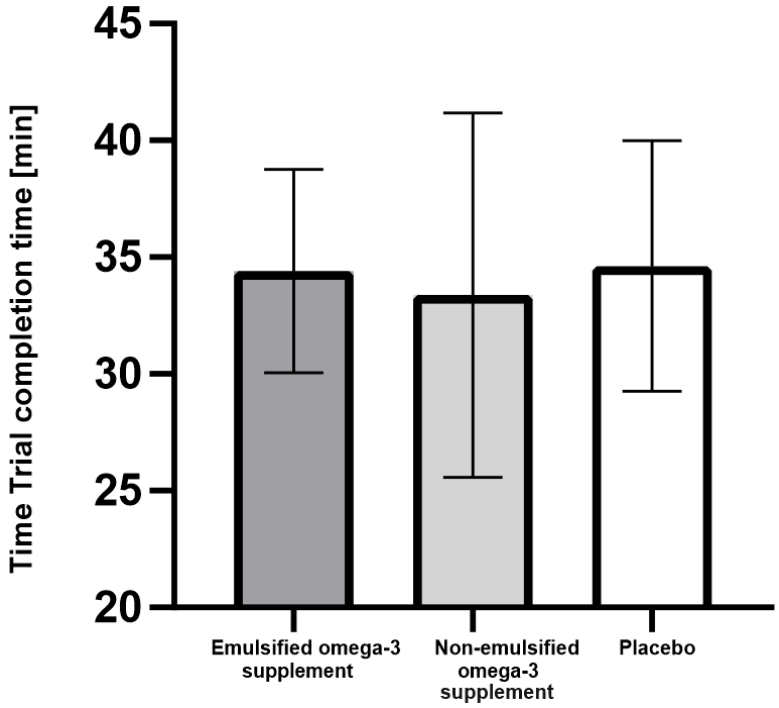


Figure 13. Average time for completion of work target time trial between interventions

It has been theorized that essential omega-3 fatty acids, EPA and DHA, could improve endurance exercise performance by affecting several mechanisms in body (Mickleborough, 2013; Williams, 2012; Peoples et al., 2008).

Cycling time trial conducted in acute state of fatigue, which places significant physiological exertion on the individual, allows examination of possible dietary interventions effects on physiological compensation during demanding exercise (Hingley et al., 2017). However, the

present study did not report improvement in time trial performance using omega-3 supplement intervention in young healthy volunteers, which is in accordance with findings from other similar studies (Hingley et al., 2017; Da Boit et al., 2015).

Perhaps other limiting factors might had influence on performance. For example, a learning effect could have been the reason for variations in participants' performance, despite one familiarisation trial. Although previous studies mostly included longer term supplementation with different types of omega-3 PUFA, maybe higher doses or longer period of supplementation would exhibit positive effects on performance improvement. Longer-term supplementation is required for metabolic adaptations and changes in substrate preference during endurance exercise, that is to increase fat utilization while sparing muscle glycogen (Philpott et al., 2019; Peoples and McLennan, 2016; Mickleborough, 2013; Peoples et al., 2008). It is possible that participants' glycogen stores were already depleted, and their muscles may have fatigued even before the time trial, which could support the observed results.

4.6. LIMITATIONS OF THE STUDY

Limited research has studied single-dose, acute supplementation with omega-3 PUFAs and its impact on endurance performance, especially in a healthy, trained population. For that reason, there are not many publications to compare the obtained results with, thus requiring further investigation. The small sample size (N = 7) gave insufficient power to better examine relationship between a dose and response, and it could be possible that some metabolic effects of fish oil omega-3 PUFAs were not detected. Also, a relatively narrow range of individuals was studied, which may limit wider applicability of our findings, particularly to women.

Although results from this study are not significant, the benefits of omega-3 PUFAs for general health are highly beneficial. Future studies should use study design with a larger sample size. It might be useful to include the detection of oxygen consumption and vasodilatory responses during exercise in the study design. Study design could also be improved by checking for markers of muscle damage and inflammation.

5. CONCLUSIONS

- Single dose of omega-3 PUFAs increased levels of EPA and DHA in blood plasma.
- No significant differences in heart rate were observed during steady state exercise after supplementing with a single dose of omega-3 PUFAs.
- No significant differences were observed in blood pressure between test supplement interventions.
- Acute supplementation with omega-3 PUFA has not been proven to improve cardiovascular function during submaximal endurance exercise.
- No time trial performance improvement was found using acute omega-3 supplement intervention in trained cyclists.
- Provision of single dose of both emulsified and non-emulsified fish oil omega-3 supplement has not been found to enhance endurance performance in trained cyclists.

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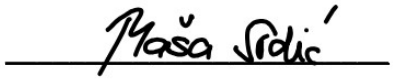
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STATEMENT OF ORIGINALITY

This is to certify, that the intellectual content of this thesis is the product of my own independent and original work and that all the sources used in preparing this thesis have been duly acknowledged.



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