Impact of two weeks pistachio supplementation on muscle recovery following downhill running

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IMPACT OF TWO WEEKS PISTACHIO SUPPLEMENTATION ON MUSCLE RECOVERY FOLLOWING DOWNHILL RUNNING

The 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 Jordan Philpott, PhD from University of Stirling.

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IMPACT OF TWO WEEKS PISTACHIO SUPPLEMENTATION ON MUSCLE RECOVERY FOLLOWING DOWNHILL RUNNING

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Abstract: Pistachios are nutrient-dense tree nuts that elicit various health benefits. There is biological rationale that pistachios could enhance recovery from intense exercise. We hypothesised that 2 weeks of pistachio supplementation would reduce the magnitude of muscle damage in response to eccentric exercise and accelerate recovery. Twenty-four participants, (27±3.4 years), undertook this randomized parallel study and were assigned to a control group (A) following their usual diet without any interventions, or groups with 42.5 g of pistachios (B), or 85 g of pistachios (C) added daily to their diet for two weeks. Following the supplementation period participants evaluated their feeling of muscle soreness (VAS), and muscle performance was assessed via vertical jump (VJ) and maximal voluntary contraction (MVC). Downhill running (DR) was performed as a muscle damage exercise bout. Muscle performance tests were repeated 24, 48, and 72 hours post DR to evaluate pistachios' impact on muscle recovery. VJ showed a decrease at 24 h post DR, but 72 h values were 0.4%, 1.9% and -0.4% of the baseline maximal jump height for A, B, and C, respectively. VAS showed a significant increase ($p<0.05$) in all monitored muscles 24 and 48 h post DR but decreased at 72h. The higher dose of pistachios seemed to contribute to a prolonged muscle soreness perception. MVC data were quite disparate and inconclusive. This study does not advocate the use of pistachios as a recovery food; however, these preliminary results require further research for the final confirmation and better understanding.

Keywords: pistachio, DOMS, muscle recovery, downhill running

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UTJECAJ DVOTJEDNOG UNOSA PISTACIJA NA OPORAVAK MIŠIĆA NAKON TRČANJA NIZBRDO

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Sažetak: Pistaciji su nutritivno bogati orašasti plodovi koji imaju razne zdravstvene koristi. Postoji biološka osnova da bi pistaciji mogli poboljšati oporavak od intenzivne tjelesne aktivnosti. Pretpostavili smo da će 2 tjedna suplementacije pistacijima smanjiti razinu oštećenja mišića do kojeg dolazi kao odgovor na ekscentričnu vježbu, te ubrzati oporavak. Dvadeset četiri ispitanika (27±3,4 godine) sudjelovali su u ovoj randomiziranoj paralelnoj studiji, a bili su dodijeljeni kontrolnoj grupi (A) koja je imala uobičajenu prehranu bez ikakvih intervencija, ili grupi s 42,5 g (B) odnosno 85 g pistacija (C) dodanih njihovoj prehrani svakog dana tijekom dva tjedna. Nakon razdoblja suplementacije, ispitanici su procijenili osjet boli u mišićima (VAS), a rad mišića je ispitan vertikalnim skokom (VJ) i maksimalnom dobrovoljnom kontrakcijom (MVC). Trčanje nizbrdo (DR) izvedeno je kao vježba kojom se izaziva oštećenje mišića. Testovi rada mišića ponovljeni su 24, 48 i 72 sata nakon DR kako bi se procijenio utjecaj pistacija na oporavak. 24 h nakon DR došlo je do pada u VJ, ali 72 h nakon vrijednosti su bile 0,4%, 1,9% i -0,4% početne maksimalne visine skoka za A, B i C grupu. VAS je pokazao značajno povećanje ($p < 0,05$) u svim promatranim mišićima 24 i 48 sati nakon DR, ali se smanjio nakon 72 h. Pri tome se činilo da veća doza pistacija doprinosi produljenoj percepciji bolova u mišićima. Vrijednosti MVC-a bile su prilično raspršene, ne ostavljajući prostora za zaključke. Rezultati ove studije ne opravdavaju upotrebu pistacija kao hrane za oporavak. Ipak, ovi preliminarni rezultati zahtijevaju daljnja istraživanja radi konačne potvrde i boljeg razumijevanja.

Ključne riječi: pistacio, DOMS, oporavak mišića, trčanje nizbrdo

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1. INTRODUCTION

Pistachio (*Pistachia vera*, L.) is a tree nut from the Anacardiaceae family that originated from Western Asia and has become one of the most popular tree nuts, mainly in middle-income countries. Over the last few years, world production and consumption of pistachio's increased markedly, and thus scientific interest, mainly with regards to potential health benefits, has garnered significant recent attention (Bulló et al., 2015). The rising awareness of healthier eating habits among consumers encouraged consumption of tree and ground nuts as a healthier snacking option (World Pistachio Report, 2019). A number of studies have explored the potential health benefits of pistachios', with application to prevention and treatment of chronic non-communicable diseases, and weight management (Terzo et al., 2017).

Pistachio nuts are recognised to be an exceptional source of vegetable protein, with a high arginine and unsaturated (especially monounsaturated) fat content, as well as phytosterols. Pistachios also constitute minerals, such as copper, magnesium, phosphorus, potassium, and calcium as well as many vitamins such as vitamins A, B, C, E and K. They are rich source of phenolic compounds, phytochemicals with antioxidant, anticancerogenic, anti-inflammatory, cardioprotective and vasoprotective properties, repeatedly confirmed through numerous studies (Hormaza and Wünsch, 2007; Bulló et al., 2015). Based on this distinguished nutrient composition, the application of pistachios in Sport Nutrition has also received interest (Nieman et al., 2014; Celik et al., 2019) specifically in terms of exercise recovery.

Exercise-induced muscle damage (EIMD) is caused by exhaustive or unaccustomed exercise and leads to negative side effects which impair athletic performance (Owens et al., 2018). Although underlying mechanisms of EIMD are complex, they are typically simplified into two phases. Firstly, initial phase of primary damage which occurs as a result of the performed mechanical work, and secondly, the secondary damage phase associated with the inflammatory response (Owens et al., 2018). There are numerous interventions proposed to promote recovery from EIMD (e.g. massage, cryotherapy, electrical therapy, medications, and nutritional interventions) (Sousa et al., 2014). From a nutritional perspective, a balanced, and individualised diet is recommended in an athlete's lifestyle. This diet provides adequate amount of all micronutrients, as well as functional food components, such as phytochemicals (Harty et al., 2019). Since data are inconclusive about supplementation strategies with regards to ameliorating muscle damage, the recommended strategy is to consume a varied diet focused on foods that are natural sources of valuable nutrients such as fruits, vegetables, and whole grains. Accordingly, the risk of exceeding intake is minimised, on the contrary to uncontrolled and possibly unnecessarily supplementation (Nieman et al., 2010).

The primary purpose of this study was to investigate the efficacy of pistachio consumption on enhance recovery from intense eccentric based exercise. We hypothesised that two weeks of pistachio consumption would reduce the magnitude of muscle damage in response to eccentric exercise and accelerate recovery.

2. THEORETICAL PART

2.1. PISTACHIO

Pistacia vera L., commonly known as pistachio, is an ancient tree nut which belongs to Anacardiaceae family of plants. Other members of the family are species such as cashew, mango, ambarella, purple mombin, poison ivy and poison oak, or pepper tree (Hormaza and Wünsch, 2007). Archaeological findings suggest cultivation and consumption of pistachio as early as 7,000 B.C. on territories of today's Turkey, Iran, and Afghanistan, making it one of the oldest flowering nut trees. Pistachio was recognised by past civilisations for its nutritional value and possible properties of disease management (Dreher, 2012; Terzo et al., 2017). By virtue of its high nutritional value and long shelf life pistachios became a popular food among early explorers and tradespeople who first spread pistachio trees from the Middle East throughout the Mediterranean basin, across North Africa, Western and Eastern Asia, and in more recent times in America and Australia (Hormaza and Wünsch, 2007). Pistachio nuts became valued delicacy both among high society and commoners (Dreher, 2012).

Until now, pistachio nuts have maintained their role as one of the most popular edible nuts worldwide. Their commercial worth is a result of their long history of cultivation and consummation, and specific sensorics characteristics such as unique colour, appealing texture, and specific flavour (Liu et al., 2014). Pistachios are usually consumed as snacks in their original kernel form raw or roasted and salted, sometimes even flavoured. On the other hand, with the emergence of the industry, they started being used as a confectionery ingredient in a wide range of food industry products such as ice creams, desserts, bakery products, and puddings, but also sauces, salads, cold-cuts, and fermented meats (Tomaino et al., 2010; Dreher, 2012; Ojeda-Amador et al., 2019).

2.1.1. Nutritional composition and health effects of pistachio

Although pistachios are known since prehistoric times as nutritionally valuable food, only recently have they begun to receive scientific interest in terms of metabolic health benefits. Most compelling evidence suggest pistachios, if consumed in moderation, provide an important part of a balanced healthy diet not only by promoting cardiovascular health, glycaemic control, and weight maintenance (Dreher, 2012) but also by preventing metabolic conditions and lowering the risk of developing chronic noncommunicable diseases (Terzo et al., 2017). Pistachios also exert a satiety effect and have been shown to lower the postprandial glycaemic response of high-glycaemic meals (Dreher, 2012).

Since nuts are nutrient- and energy-dense food, it is often believed that including them in the diet will result with the weight gain. However, data suggest otherwise, even opposite (Li et al., 2010). Pistachios are reported to successfully be part of a weight reduction diet, without concern that they will cause weight gain but only if consumed controllably. Compared to isocaloric snack of pretzels, as part of reduction diet, even greater weight loss was observed, as well as reduction in triglycerides (Li et al., 2010). In healthy woman in a free-living setting, impact of an afternoon snack was examined. For four weeks, pistachios and biscuits were tested and resulted in similar post-snack food intake and feeling of satiety. Neither type of an afternoon snack had impact on body weight, but important to mention that pistachio group improved their micronutrient intake, especially for vitamins B1 and B6, cooper, and potassium (Carughi et al., 2019). It has been reported that nuts provide less metabolised energy in vivo than calculated by proximate analysis and standardised Atwater factors (Baer et al., 2012; Carughi et al., 2019), which could be one of the reasons that caloric contribution to the diet is overestimated. Another positive aspect of pistachio consummation on reduced calorie intake is their in-shelled form. It has been proven that children who eat more slowly, consume fewer calories, which is also the case with adults. Slower food consumption results in higher levels of satiation and reduced calorie intake. Food intake could be decelerated with food or packaging characteristic, or affection in perception of food volume, such as leaving visual cues. Since pistachios are available for purchase both in in-shelled and shelled form, selecting in-shell nuts may result in lower calorie consumption, but with same satiety levels (Honselman et al., 2011).

The beneficial effects of pistachios consumption on overall health have been attributed to their macro- and micronutrient, and phytochemical profile. Pistachios contain high concentrations of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in combination with low saturated fats (SFA), dietary fibre (both soluble and insoluble), quality plant protein, minerals such as magnesium and potassium, and vitamins B, E and K. Moreover, phytochemicals, including tocols (γ-tocopherol), phytosterols (stigmasterol and campesterol), carotenoids (lutein and zeaxanthin), chlorophyll, and polyphenols (flavonoids, anthocyanidins, etc.), may also play an important part to a variety of pistachio’s effects through their antioxidant, anti-inflammatory, antimicrobial, photoprotective, and antimutagenic characteristics which all act synergistically (Dreher, 2012; Liu et al., 2014; Bulló et al., 2015; Ojeda-Amador et al., 2019). Because of these bioactives pistachios were included among the top 50 foods with the highest antioxidant potential (Dreher, 2012; Liu et al., 2014; Bulló et al., 2015; Ojeda-Amador et al., 2019).

Pistachios' phytochemicals such as polyphenols, xanthophylls and tocopherols, have been confirmed as easily accessible in the stomach consequently increasing their absorption capacity in the small intestine. Along with greater absorbed amounts, impact of pistachio consumption on health is potentiated (Bulló et al., 2015). However, bioacessibility of nutrients during the digestion has yet to be confirmed given the inconclusive results. Kay et al. (2010) reported that consumption of 10% (32−63 g/d) or 20% (64−126 g/d) of total daily calories intake from California pistachios for 4 weeks elevated levels of circulating β-carotene, lutein, and γ-tocopherol in hypercholesterolemic adults, which suggest good bioavailability of lipophilic compounds. Moving forward, more studies are warranted to verify the bioaccessibility and bioavailability of other bioactive compounds, and possibly compare different pistachio varieties (Kay et al., 2010). Liu et al. (2014) utilized the in vitro gastrointestinal (GI) digestion mimic to evaluate the bioaccessibility of the lipophilic and phenolic compounds. Most compounds were found not to be easily accessible during the digestion. From lipophilic compounds, only lutein and γ-tocopherol were mensurable in GI mimic extract, however, with concentrations 93 and 95% lower than previously quantified concentrations using organic solvents. These compounds were detected only in nutmeat and whole pistachios extract but not in the skin. Regarding phenolic compounds, only gallic and protocatechuic acids were quantifiable in the nutmeat GI digest extract, while in the skin 9 out of 11 compounds were detected with concentrations 34-94% lower than in the acidified methanol extracts. In whole pistachios 7 compounds were detected with more than 90% lower content than in the acidified methanol extract (Liu et al., 2014). On the contrary, Mandalari et al. (2013) showed more than 90% bioaccessibility of pistachio compounds such as gallic and protocatechuic acids, lutein, eriodyctiol, tocols and lutein, without differences between raw shelled or roasted salted pistachios (Mandalari et al., 2013). Serafini et al. (2010) reported that polyphenols content in food cannot be reflected by plasma concentrations. Human intervention studies have shown that absorption of flavonoids in the gastrointestinal tract is in general between 1 and 5% of the ingested dose (Serafini et al., 2010) which is consistent with Liu et al. (2014) report.

Pistachios are, like other nuts, a rich source of fat. However, they contain mostly unsaturated fatty acids (MUFA and PUFA) with a balanced ratio. MUFA and PUFA have positive effect on health if consumed in moderation (Ojeda-Amador et al., 2019). It has been established that the type of fat, rather than the total amount of fat is responsible for reduced risk of cardiovascular diseases, and favourable effect on blood lipid status such as adequate serum cholesterol levels (Ryan et al., 2006). At the same time, quantity of fat in pistachios is lower than in other nuts, consequently caloric value is also lower. On the other hand, they have greater amounts of dietary fibres, and antioxidant and anti-inflammatory phytochemicals. Such a nutritional profile distinguishes pistachios among other nuts and propose huge potential of its consummation in prevention of dysmetabolic conditions (Bulló et al., 2015; Terzo et al., 2017). It has been stated that people consuming more than 7,09 g/d of nuts or nut products such as tree nut butters, have significantly higher intake of fibre, vitamins and minerals, and higher Total Healthy Eating Index-2005 score (Bulló et al., 2015).

In 2003, Food and Drug Administration (FDA), authorized a health claim about the relationship between the consumption of nuts and reduced risk of cardiovascular diseases (CVD). The permission has been given to pistachio nuts producers to state the following claim on their products: "Scientific evidence suggests but does not prove that eating 1.5 ounces per day of most nuts, such as pistachios, as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease." The claim is applicable to almonds, Brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pecans, pine nuts, pistachio nuts, and walnuts. However, some types of these nuts exceed the saturated fat levels, so more attention is needed to adequately label these products. The claim is also applicable only to "whole or chopped nuts" but not to "nut-containing products" (FDA, 2003).

2.1.1.1. Energy value

Hamasaki and Hamasaki (2017) in their review article stated that pistachio nuts contain 557.4 kcal/100 g, with only cashews and peanuts containing less energy. However, it seems that pistachio nuts contain 5% less energy content than currently accepted value which was calculated from the Atwater general factors. Measured energy of pistachio is 633 kJ/28 g serving, or 22.6 kJ/g that is, that is 151.3 kcal/28 g of serving and 5.4 kcal/g, respectively. The reason lies behind that all nutrients are not bioaccesible and digested in total. Accurate information about energy values are important for reliable food labelling and proper informing consumers (Baer et al., 2012). Dreher (2012) reported value of 159 kcal/28.35 g of pistachios, same as Nieman et al. (2014), Bulló et al. (2015) and Terzo et al. (2017). According to Bulló et al. (2015), pistachios have the least energy among almonds, hazelnuts, macadamia nuts, peanuts, pecans, and walnuts. Nutrition facts per 28 g of pistachios could be seen in Figure 1.

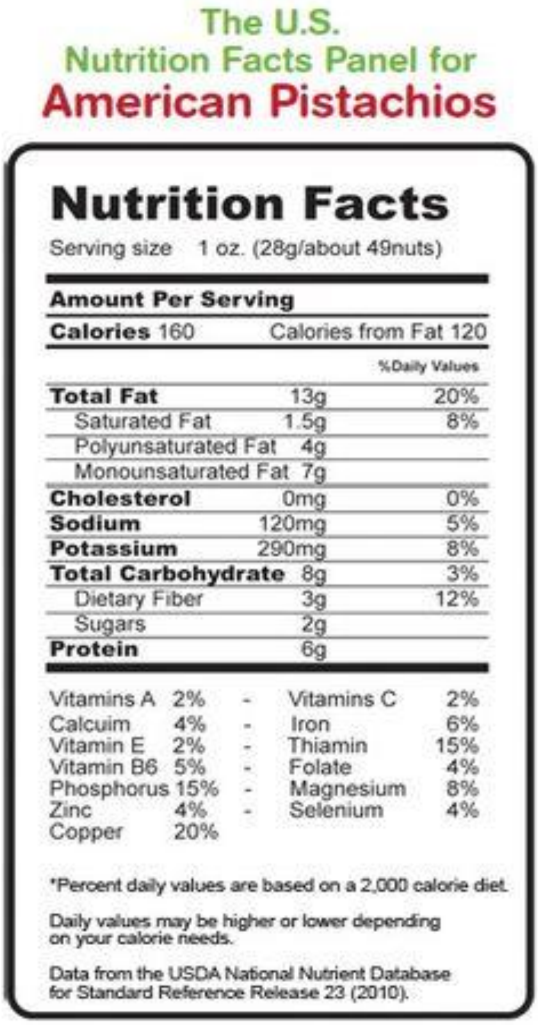

Figure 1. Nutrition facts for 28 g of pistachios (source: americanpistachios.org)

A prospective study conducted in Turkey showed that adding 20% of total energy intake in form of pistachios to Mediterranean diet compared to Mediterranean diet alone, improve blood glucose level, endothelial function, and some indices of oxidative stress and inflammation in healthy young men (Sari et al., 2010). Similar was confirmed in healthy woman in the USA. Inclusion of 20% of total calories as pistachios did not contribute to weight gain, body fat or blood lipid changes but improved diet quality (Burns-Whitmore et al., 2017). However, addition of any food is not recommended to non-healthy or overweight persons.

2.1.1.2. Carbohydrates and fibre

The overall amount of carbohydrates in pistachios is considered low to moderate with approximate value of 29 g/100 g reported by Terzo et al. (2017) or 27.5 g/100 g (Nieman et al., 2014; Bulló et al., 2015) of which 7.65 g is derived from naturally occurring sugars, 1,66 g from starch and 10.34 g from fibre (Nieman et al., 2014). Regarding fibre, pistachios are richer than other nuts with 10 % and 0.3% of their weight in insoluble and soluble forms, respectively (Terzo et al., 2017). That amount of fibre correspond to 3 g/28.35 g or 12% of Recommended Dietary Allowance (RDA) per serving basis. The importance of fibre content is demonstrated through epidemiological and clinical studies which inversely associate it with weight gain, cardiovascular diseases, diabetes, and some types of cancer (Bulló et al., 2015). Additionally, pistachios are considered as low glycaemic index food and can even lower postprandial blood glucose fluctuations which contributes to maintenance of prolonged satiety (Terzo et al., 2017). When eaten alone, pistachios have a minimal effect on blood glucose levels, yet if consumed with a carbohydrate rich meal, they manage to diminish the postprandial glucose response (Kendall et al., 2011). Pistachios have a very low glycaemic index that ranges from 4 to 9. It has even been reported that adding pistachios to foods with high glycaemic index (e.g. pasta, parboiled rice, or potatoes) could reduce overall postprandial glycaemic response by 20-30% (Dreher, 2012). It is interesting that reduction in white bread glycaemic response is dose dependent. However, care must be taken not to increase calories above one’s requirement and nuts should be substitution for other foods and not simply added to the diet (Kendall et al., 2014).

Data reports indicate that sucrose is the predominant sugar in pistachio nuts, although some others identified glucose as principal one, possibly due to sucrose hydrolysis (Kader et al., 1982). Additionally, they are source of raffinose, a soluble trisaccharide also found in onions and majority of legumes. In plant, raffinose has many useful functions such as transport, storage, and cryoprotection. In humans, raffinose is poorly absorbed due to lack of adequate enzymes, but it is an effective prebiotic, stimulating the growth of favourable colon microflora

(Nieman et al., 2014). They also contain phytates in variable concentrations from 0.29 to 2.83 g/100 g (Nieman et al., 2014).

2.1.1.3. Protein

According to MyPlate, nuts, such as pistachios, are part of the Protein Foods Group. ½ ounce of nuts (corresponding to 24 pistachios) is considered as 1 ounce-equivalent from Protein Foods Group. Both men and women in age groups 19-30 and 30-51 years are recommended to consume 6½ and 6 oz-equivalent amount of protein foods, respectively. Active people (more than 30 minutes per day of moderate physical activity) could consume more as long as they stay in their required calorie intake/are not exceeding their required calorie needs (USDA/MyPlate, 2020).

Pistachios are a valuable source of vegetable protein which make up approximately 20% of their total weight gain, that is 20.27 g/100 g or almost 6 g per serving (28.35 g) (Nieman et al., 2014). They contain all of the essential amino acids in sufficient amount, with lysine as the first limiting amino acid (Terzo et al., 2017). According to the Protein Digestibility Corrected Amino Acid Score (PDCAAS) pistachios are source of all nine essential amino acids, and have score of 81% of casein, which is a reference protein food which is more than quinoa, chickpeas, soybeans, cooked kidney beans and roasted peanuts (American Pistachio Growers, 2020).

Furthermore, they are good source of arginine and branched-chain amino acids (BCAA). L-arginine is a precursor of nitric oxide (NO) which is an important molecule because of its vasodilator properties and as such involved in the cardiovascular system. In pistachios could be found in concentrations about 9.15 g/100g (Terzo et al., 2017). BCAA are provided by pistachios in higher amounts than by other tree nuts. They contain 1.599 g leucine, 0.932 g isoleucine and 1.262 g valine per 100 g. Furthermore, they have essential amino acid ratio (essential amino acid:total amino acid) of 39.1, which is higher than most of other generally consumed tree nuts like almonds, walnuts, pecans or hazelnuts (Bulló et al., 2015).

2.1.1.4. Fat content

Even though nuts are considered as high-fat foods, pistachios, compared to other nuts, are relatively poor in fat, containing only 45.4 g /100 g. Pistachios fat content is mainly composed of MUFA (23.8 g/100 g) and PUFA (13.7 g/100 g). At the same time, the saturated fat content is very low, only 5.6 g/100g (Nieman et al., 2014). More than 70% of the total fat amount is represented by oleic and linoleic fatty acids (Terzo et al., 2017). Major MUFA present in five different types of nuts (brazil, pecan, pine, pistachio, cashew) was oleic acid (18:1), and it was particularly high in pistachio and cashew. Most abundant PUFA was linoleic fatty acid (18:2) in the same set of nuts. Primary SFA were palmitic (C16) and stearic (C18) acids. Pistachios and cashew were only nuts containing more MUFA than PUFA, with pistachio containing almost 60% MUFA of total fats. Among five nuts, pistachio and cashew had lowest amount of total oil and highest peroxide value (Ryan et al., 2006).

2.1.1.5. Vitamins and Minerals

Pistachios are micronutrient-dense food. They contain almost all vitamins that is vitamin A, vitamin E, especially in the form of γ-tocopherol, vitamin K, vitamin C and B vitamins, with exception of B12 (cyanocobalamin) (Bulló et al., 2015; Terzo et al., 2017).

Pistachio nuts are rich in thiamine, vitamin B1, which is involved in intermediary carbohydrate metabolism (Bulló et al., 2015; Terzo et al., 2017). The amount of it they contain is 0.87 mg/100g of pistachios which correspond to 50% of the RDA value. Amount of pyridoxine, vitamin B6, even exceeds RDA value with 1.7 mg/100 g, while amount of folic acid, vitamin B9, present is around 25% of the RDA (Bulló et al., 2015). In the human body, pyridoxine is involved in the metabolism of amino acids and production of niacin, while folic acid is required for the structural proteins and haemoglobin formation (Terzo et al., 2017). Compared to other nuts they stand out for high vitamin K concentrations with approximately 13.2 mg/100 g or 16% of the RDA (Bulló et al., 2015). Higher dietary intake of vitamin K has been associated with a lower risk of chronic diseases such as diabetes type 2, cancer, and cardiovascular diseases (Bulló et al., 2015; Terzo et al., 2017).

Likewise, they are rich in minerals, especially potassium which they contain in the highest amount amongst other nuts. They are also rich in zinc, selenium, magnesium, manganese, calcium, cooper, and phosphorus. This mineral profile allows pistachios to play an important role in blood pressure regulation, in bone-related diseases and prevention of chronic diseases (Bulló et al., 2015; Terzo et al., 2017). Study with rats showed that wild pistachio extracts consumed while endurance training could prevent from reduction of liver micronutrients such as calcium, zinc, and cooper (Yousefi et al., 2013).

2.1.1.6. Phenolic compounds

Pistachios, as well as pecans and walnuts, are great sources of multifarious phenolic compounds, including anthocyanins, proanthocyanidins, flavan-3-ols, flavonoids, flavonols, isoflavones, flavanones, stilbenes, phenolic acids, and hydrolysable tannins. All of these are exceptional as antioxidants which coupled with their chemopreventive, cardioprotective and vasoprotective properties make them important part of the human diet (Tomaino et al., 2010; Bulló et al., 2015).

Ojeda-Amador et al. (2019) conducted a comprehensive study in which they examined differences in the phenolic profile of eight different pistachio cultivars. All cultivars were grown under the same agronomical conditions. In total, 26 compounds were found, and 25 of them identified, in all eight cultivars. Three anthocyanins, ten flavonols, one gallotannin, nine flavanols and two flavanones were detected, some of them first time reported to be present in pistachio nut. Although all varieties had the same qualitative profile, significant differences were perceived regarding quantity (Ojeda-Amador et al., 2019). Some other authors reported less diverse phenolic profile. Tomaino et al. (2010) identified 10 phenolic compounds in pistachio nutmeat, and 13 in the skin, in total 17 different phenolics while Liu et al. (2014) identified only 11 of them (Tomaino et al., 2010; Liu et al., 2014).

Larnaka variety was cultivar with the highest overall phenolic content (4893 mg/kg), while Kerman was one of the cultivars with the lowest phenolic concentration (1936 mg/kg) (Ojeda-Amador et al., 2019). According to Yang et al. (2009), total amount of phenols was even higher with 571.8 mg/100 g, including bounded and free phenols. Out of nine tree nuts and peanuts, only walnuts, pecans and peanuts contained more phenolics than pistachios (Yang et al., 2009). All phenolic compounds found in pistachio are present in greater amounts in skins compared to seeds. To demonstrate, total flavonoids content in skins is 70.27±5.42 mg (expressed as Catechin Equivalents/g of fresh weight), while in seeds it is merely 0.46 ±0.03 mg Cat E/g f.w (Tomaino et al., 2010). Flavanols were confirmed to be the most abundant phenolic group present in pistachios contributing approximately with 90% of the overall phenolics content. Predominant compounds were catechin, epicatechin, gallocatechin, procyanidin B1 and B2, and proanthocyanidin dimer. Procyanidin B1 and catechin were two major flavanol compounds with highest concentrations in Larnaka variety, while Kerman was one of the cultivars with the lowest concentrations of both. Catechin is known as one of the most powerful antioxidants among flavanols (Ojeda-Amador et al., 2019).

Kerman variety had highest concentrations of flavonols which were represented by glycosides of quercetin, myricetin and kaempferol and free quercetin (Ojeda-Amador et al., 2019). Liu et al. (2014) stated that pistachio nutmeat is rich in luteolin and myricetin (Liu et al., 2014). Quercetin is one of the selected immunonutritional supplements which could exert positive effects in exercise-stressed athletes. The dose suggested is 1000 mg/day for two to three weeks to reduce illness rates. Nevertheless, it seems that combinations with other polyphenols or food ingredients, for example, green tea extract, fish oil or isoquercetin, successfully nullify negative impact of strenuous exercise and enhance innate immunity function (Walsh et al., 2011). The amount of quercetin and its derivates in Kerman pistachio was around 79 mg/kg fresh weight (Ojeda-Amador et al., 2019).

Tomaino et al. (2010) stated that pistachio skin constitutes about 10% of the total shelled pistachio weight, while Liu et al. (2014) determined that value to be 6%. Moreover, they suggested that pistachio skin is the most responsible for the high antioxidant activity of the whole nut (Tomaino et al., 2010). In particular, skin, unlike nutmeat, contains some other phenolic families such as anthocyanins (Ojeda-Amador et al., 2019). For that reason, pistachios are unique among tree nuts since they are the only nuts containing anthocyanins (Bulló et al., 2015). Three different anthocyanins were detected in pistachio skin, with cyanidin-3-O-galactoside being the most abundant one. Kerman variety had the highest concentration of cyanidin-3-O-glucoside, but the lowest total anthocyanin content (Ojeda-Amador et al., 2019). Other phytochemicals which seems to be responsible for antioxidant activity of pistachio skin include gallic acid, catechin, quercetin, cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, eriodictyol-7-O-glucoside and epicatechin (Tomaino et al., 2010; Liu et al., 2014). Pistachios were on the 4th place regarding the total antioxidant activity, expressed as micromoles of vitamin C equivalents per gram of sample, behind walnuts, pecans, and peanuts (Yang et al., 2009). Kerman and Kastel were varieties with the smallest Trolox Equivalent Antioxidant Capacity (TEAC) values, while Larnaka had the highest one (Ojeda-Amador et al., 2019).

Anthocyanins are red, purple, black or blue pigments found in many fruits and vegetables, and their products such as grapes, red wine, cherries, blueberries, red oranges etc. They are reported as possible antioxidant, anti-inflammatory, anticarcinogenic, and antiangiogenic agents (Tomaino et al., 2010). Extrapolated value of total anthocyanin content expressed as cyanidin-3-O-glucoside in the whole pistachio nut is 0.50 mg/g. Since that value varied a lot from other reported values it was concluded that anthocyanin content may be affected by cultivar, geographical origin, ripening stage, and industrial processing factors (Tomaino et al., 2010). Catechins, most represented in the tea, are demonstrated to be the most effective in reducing the oxidation of low-density lipoprotein (LDL) and thus preventing cardiovascular diseases (Tomaino et al., 2010). Isoflavones, for instance genistein, showed possibility of reducing the incidence of some cancer, especially estrogen-related ones such as breast and uterus cancer, since they act as partial agonist of the estrogen receptors (Tomaino et al., 2010).

Since there is much evidence that fruits and vegetables originated polyphenolic antioxidant compounds have preventative role in cancer, inflammation, and cardiovascular disease conditions, adding pistachios to regular diet could be extremely useful in maintaining health and prevention of chronic diseases. It has been advised to consume unpeeled pistachios rather than peeled ones due to higher nutritional value and stronger health beneficial effects. Pistachio skin, as a significant industrial by-product, if removed from the nut should find its way to cosmetic and pharmaceutical industry. Otherwise, it could become waste and if not disposed properly, possible to be part of the environmental pollution (Tomaino et al., 2010).

2.1.1.7. Carotenoids, chlorophylls, and phytosterols

Pistachios are only nuts with considerable xanthophyll carotenoid content. The same is represented by lutein and zeaxanthin, with concentration of 1160 μg of lutein + zeaxanthin/100 g (Terzo et al., 2017). Bulló et al. (2015) proposed even greater content of 1405 μg of lutein + zeaxanthin/100g, what is about fifteen times more than in hazelnuts, which are the next highest type of the nut with 92 μg/100 g. These two carotenoids are mainly responsible for unique green pistachio colour (Bulló et al., 2015). Moreover, among tree nuts, only pistachios and pine nuts contain chlorophylls. Similar concentrations of chlorophylls are found in pistachios harvested in Italy, Turkey, Greece, Iran, and California (Liu et al., 2014). Furthermore, pistachios have the highest phytosterol concentrations of 289 mg/100 g according to Terzo et al. (2017) or similarly 214 mg/100g according to Bulló et al. (2015). These are represented by stigmasterol, campesterol and β-sitosterol (Bulló et al., 2015; Terzo et al., 2017). Phytosterols are similar in structure to cholesterol, hence are included in mediation of a dose-response reduction of cholesterol concentrations. Regardless insufficient phytosterols amount per serving to support FDA health claim, it is believed that synergistic effect with low SFA levels and high UFA levels help to maintain adequate cholesterol levels (Bulló et al., 2015).

Liu et al. (2014) for the first time quantified γ-tocotrienol, zeaxanthin, α-carotene, and myricetin in Californian pistachios. On the whole, they characterized 9 lipophilic compounds (four carotenoids, two chloropylls, and three tocols). They identified lutein as dominant carotenoid, chlorophyll a as main chlorophyll and γ-tocopherol as representative of tocols (Liu et al., 2014). Pistachio had the highest γ-tocopherol content, and the highest total phytosterols content among Brazil nut, pecan, pine nut and cashews (Ryan et al., 2006).

2.1.2. Pistachio plant

Pistachio tree is deciduous, dioecious, wind-pollinated, and flourish desert tree used to hot climate and highly tolerant of saline soil. It can grow up to 10 meters high. Its fruits, pistachio nuts, grow in heavy grape-like clusters surrounded by a fleshy hull. Depending on a variety, during late summer or early autumn, hulls become rosy and inner pistachios' shells split spontaneously, that way signifying the ripening stage. The nut itself has a hard, cream coloured shell inside which is a light green kernel covered with thin purplish skin (Hormaza and Wünsch, 2007; Dreher, 2012). *Pistacia* belongs to tribe Rhoeae, one of five tribes of Anacardiaceae family. It is considered that *Pistacia* comprise of 11 species divided into four sections, but some authors recognise even 15 species. It is considered that *P.vera* has a high degree of genetic vulnerability because only few primitive varieties are parental to less than 100 cultivars of *P.vera* worldwide (Hormaza and Wünsch, 2007). Costa et al. (2017) stated that there are at least 12 different *Pistacia* species, some of them with additional subspecies. While *P. vera* is economically most interesting because of its edible nuts, other species are used with various purposes, such as for materials, fuels, medicine or as gene source (Costa et al., 2017). Recently, breeding programs started in different pistachio producing countries, to produce more desirable product (e.g. increased percentage of split shells, reduction of empty nuts, increased yields, increased nut size and appearance, resistance to diseases and environmental conditions) (Hormaza and Wünsch, 2007).

Varieties cultivated in different countries could significantly vary in nutritional composition of the nut. For example, US pistachios have lower energy value and higher amounts of carotenoids lutein and zeaxanthin. On the other hand, Iranian varieties contain higher amounts of linoleic acid, while Turkish ones have higher levels of calcium. Temperature under which pistachios are grown could affect type and level of fatty acids. For instance, pistachios grown in temperatures over 25°C tend to have lower amount of saturated fats like palmitic fatty acid (Satil et al., 2003). It has been reported that wide alterations in phenolic concentrations in plant foods are possible due to influence by growing location, season, cultivar, and food processing (Liu et al., 2014). In the same way, some post harvesting processes (e.g. drying, roasting, or bleaching) could cause degradation of phytochemicals in pistachio skin (Tomaino et al., 2010). Factors defining micronutrient content and sensorics characteristics include cultivar and cultivation and cultural practices, climatic conditions, harvest season, irrigation, soil, growing region, and maturity level. Equally important are postharvest processing, transport manners and storage methods (Kader et al., 1982; Liu et al., 2014).

In a study from 1982, Kerman variety kernels (Californian pistachio) were rated higher in firmness and sweetness, and lower in crispness, bitterness, and rancidity than some other cultivars. The best quality nuts are harvested at near optimum maturity, early September, and dried to 4-6% moisture level. Dried pistachios could be kept for 12 months at 20°C (Kader et al., 1982).

2.1.3. World production and consumption

Among the nut tree crops, pistachio tree was ranked sixth in world production behind almond, walnut, cashew, hazelnut and chestnut (Hormaza and Wünsch, 2007), but increased over the years and Costa et al. (2017) reported, according to 2017 FAOSTAT, that pistachios ranked third, behind almond and cashews (Costa et al., 2017). This is in accordance with the International Nut and Dried Fruits Council Foundation (INC) statistical yearbook in which is stated that in 2016, pistachios ranked 5th and 3rd most consumed tree nut in high-income and middle-income economies, respectively (INC, 2018).

According to the Food and Agricultural Organization (FAO) STAT database, for years Iran and the USA have been two major world pistachio crop producers which make up more than 70% of world production. In 2018, Iran alone produced 551,307 tons of pistachios while in the USA, rather California as its leading producing state, 447,700 tons were produced. They were followed by Turkey (240,000 t) and China (74,828 t) summing up to more than 95% of world production whereas only 1,5% was based in Mediterranean European countries with Greece (8,558 t), Spain (8,277 t) and Italy (3,864 t) as major producers (FAO, 2020).

Over the period from 2009-2012, the average pistachio world consumption was 475,000 metric tons per year. Iran and the USA as two largest producers are also main exporting countries, while the EU is the world's largest importing region (Xiong, 2017). As stated in the article from 2018, consumption of pistachios had increased significantly on international level between 2015 and 2017. This global interest in pistachio consumption could be due to rising evidence of beneficial effects of pistachio intake on health and weight management. The USA, as one of the two largest producers in the world, export approximately 70% of its yield primarily to China, South Korea, Germany, France, Italy, Spain, and the United Kingdom (Eddy, 2018; American Pistachio Growers, 2018). Californian pistachios are the highest valued specialty crop exported to China. Despite the trade war between the two countries, pistachio exports to China more than doubled only from 2017 to 2018 (World Pistachio Report, 2019). Furthermore, pistachio consumption in China met the 182.4% increase over those three years [2009-2012] (Eddy, 2018; American Pistachio Growers, 2018).

According to WorldAtlas and INC, in 2016 Turkey was the largest consumer country of pistachios with 130,202 metric tons consumed. In other words, such consumption means 1.64 kilograms of pistachio per capita per year. In Turkey, pistachios are consumed as snack or as confectionery ingredient for ice-creams and bakery products. Pistachios are used as a filling for one version of traditional Turkish sweet dessert pastry, baklava. The USA and Iran are second and third largest consumer countries, respectively (Misachi, 2018; INC, 2018).

Market for healthy snacking alternatives expanded as people become more aware of benefits of an active lifestyle and healthier diet. Pistachios could find their niche in that segment thanks to various reasons such as unique sensorics characteristics and beneficial health impact (American Pistachio Growers, 2018). They could also find their place in the diet of recreational and professional athletes. Not only they are good grab-and-go snack for themselves, there are already several sport brands offering sport bars containing pistachios or with pistachio flavour.

NAMEDSPORT® offer both protein and energy bar with pistachios, while Power Pro, NUTREND®, ACTIVLAB and AMIX™ offer low carb, protein bars.

2.1.4. Processing and storage

In the same way as other nuts, pistachios are often processed using common methods like roasting and steam roasting in order to enhance safety and palatability. Equally important, these processing methods intensify flavour, colour, texture, and overall appearance of pistachios (Bulló et al., 2015). Changes that occur during roasting are extremely favourable for increasing the desirability of the final product (Nikzadeh and Sedaghat, 2008). On the other hand, food processing could have negative impact on micronutrient composition since high temperatures cause phytochemicals reduction and vitamin loss. Reduction of some bioactive compounds could go up to 90% after the exposure to 160°C for 40 min (Bulló et al., 2015). Still, it has been reported that nuts exhibited their best quality when roasted at 120-160°C (Hamasaki and Hamasaki, 2017). Regarding packaging, modified atmosphere showed as more efficient than vacuum and conventional methods and is generally recommended for the maintenance of quality of fresh raw pistachios for longer storage periods (Ozturk et al., 2016).

Together with processing and packaging methods, adequate storage plays an important role in maintaining pistachios quality and safety. Low storage temperature, such as 10°C, is the most important condition, but some others like humidity and ventilation will assure quality maintenance as well as absence of mould and bugs (Bulló et al., 2015). Pistachio nuts could be source of aflatoxins, a highly toxic mycotoxin produced by *Aspergillus* fungus. According to EFSA 8-10 μg/kg of aflatoxins in pistachio nuts is maximal allowed concentration (Costa et al., 2017). The 2019 World Pistachio Report stated Codex's maximum aflatoxin level for "ready-to-eat" tree nuts is 10 μg/kg, while for tree nuts intended for further processing that level is 15 μg/kg (World Pistachio Report, 2019).

In addition to positive effects of pistachio processing, it is important to mention that heat induces protein denaturation, which is notable because pistachios, same as other nuts, contain some protein allergens which may trigger hypersensitivity reactions type I. These proteins are heat-sensitive and after denaturation lose their IgE-binding activity (Bulló et al., 2015).

2.1.5. Allergenicity

Tree nuts are among the eight foods that cause the majority of allergic reactions to foods in Europe and the USA. Additionally, they are primarily responsible for fatal allergic reactions in the USA and the UK. Their presence in the food must be indicated on the label (Sanchiz et al., 2018). Pistachio and other tree nuts allergies are increasing as a result of changes in food habits and rise in the nut consumption (Costa et al., 2017).

According to conducted studies regarding pistachio nuts allergies, it was estimated that 27% of 317 children/adolescents in France with already reported food allergies had pistachio or cashew nut allergy, based on a questionnaire. In Sweden, prevalence of pistachio nut allergy was 2% among children admitted to paediatric hospital because of acute food allergy reactions. In Finland, prevalence was 14% or 55% in subjects without birch sensation or with concomitant birch sensation, respectively. It has also been reported that prevalence of pistachio allergy is higher for South Asian than white children, since pistachio is more present in Asian cuisine. The prevalence ratio of pistachio nut reactivity in subjects with or without sensitisation to birch pollen was lower compared to hazelnut, almond or peanut. In the USA in 2008, prevalence of tree nut allergy was 1.4%, out of which 9.8% for pistachio (Costa et al., 2017). Allergic reactions to pistachios are typically immediate and induce moderate to severe clinical symptoms. Clinical manifestations include hives, vomiting, abdominal pain, nasal congestion, angioedema, urticaria, pruritus, itchy throat, repetitive coughing, wheezing, red/watery eyes, dyspnoea, erythema, eczema, lip swelling and hypotension (Costa et al., 2017)..

Since they belong to the same family, cross-reactivity between pistachio, cashew, and mango has been observed. Similar to other common food allergens, food processing can modify the structure and function of proteins and may alter (by increasing or decreasing) their allergenic properties. The knowledge about the effects of food processing on the allergenicity of pistachio nut is still very limited (Costa et al., 2017) but data suggest that in the pistachio and cashew case, heat and pressure treatments are able to decrease their IgE-binding properties. However, tested treatments usually are not applicable in real life situations because of its cost or duration. With this in mind, allergic persons are still recommended to carefully read labels and avoid products containing pistachios (Sanchiz et al., 2018).

2.2. EXERCISE INDUCED MUSCLE DAMAGE

Exercise-induced muscle damage (EIMD) is caused by exhaustive or unaccustomed intensive exercise. Its appearance leads to several undesirable side effects which impair athletic performance (Owens et al., 2018). Symptoms typically present immediately after demanding exercise bout and last up to 14 days depending on the type, intensity, and duration of damaging exercise, together with the athlete's susceptibility to the stimulus. Symptoms include ultrastructural muscular disruption, delayed onset muscle soreness (DOMS), localised oedema, inflammation, decreased range of motion, impaired muscle force producing capacity, and elevated circulation levels of specific intramuscular proteins and enzymes such as myoglobin, creatine kinase, and lactate dehydrogenase. Moreover, increases in markers of inflammation such as C-reactive protein and various interleukins, were recorded (Sousa et al., 2014; Harty et al., 2019; Owens et al., 2018). Modes of exercise that usually result in these symptoms include resistance training, prolonged or downhill running, and intermittent, high intensity exercise (Owens et al., 2018). In other words, damaging exercise is defined as any type of exercise which significantly trigger a primary outcome measure associated with EIMD, such as soreness, muscle function, serum markers of muscle damage, or cytokines (Harty et al., 2019).

Although the underlying mechanisms of EIMD are complex, they are usually simplified into two phases. First, initial phase of primary damage occurs as a result of the performed mechanical work, and second, the secondary damage phase that proliferates tissue damage through processes associated with the inflammatory response (Owens et al., 2018). There are numerous interventions trying to promote recovery following the EIMD (e.g. massage, stretching, cryotherapy, electrical therapy, medications such as non-steroidal anti-inflammatory drugs (NSAIDS), and nutritional interventions) (Harty et al., 2019). Despite the detrimental effects of EIMD, inflammation and increased protein turnover are essential for muscle adaptations and hypertrophy. However, there may be a threshold beyond which excessive damage could impair athletes' ability to train, and promote risk of injury (Sousa et al., 2014).

Since discomfort caused by EIMD can be significant and could impair subsequent athletic performance or training quality, particularly in athletes and active individuals who require rapid recovery between demanding training sessions or competitions, the development of effective nutritional and supplementation strategies to combat EIMD is of paramount importance to athletes, coaches, dieticians, researchers, and fitness professionals (Harty et al., 2019).

A wide assortment of ingredients, functional foods, and dietary supplements have been investigated by researchers in the context of reducing muscle damage, with contrasting results. Initial evidence suggests that the long-term consumption of antioxidant-rich foods (e.g. tart cherry juice, pomegranate juice, beetroot juice, and watermelon juice) as well as several chronic supplementation strategies (e.g. creatine, Ω-3 polyunsaturated fatty acids, and vitamin D3) may help to mitigate symptoms of EIMD and improve muscle function in a variety of populations (Harty et al., 2019). Moreover, many emergent strategies showed some promising results but have not been fully explored and explained, including black tea-sourced polyphenols, blueberries, chondroitin sulphate, high chlorogenic acid coffee, fasting, garlic, leucine metabolites such as HICA, lemon verbena, lychee, mate tea, pequi fruit, quercetin, saffron, selenium, sesame, spinach, and tomato juice (Harty et al., 2019).

2.2.1. Delayed Onset Muscle Soreness

Delayed onset muscle soreness (DOMS) is a phenomenon that occurs in both athletes and non-athletes when they are repeatedly exposed to high force eccentric or unaccustomed muscle contractions. During eccentric exercise, less active muscle fibres that are more prone to damage are required to produce higher muscular forces which lead to the potential injury mechanisms (Hotfiel et al., 2018). DOMS is a set of symptoms accompanied by a reduction in exercise performance such as muscle strength and range of motion (ROM). Moreover, it brings continual and prolonged physical discomfort (Kim and Lee, 2014) and it is associated with increased creatine kinase (CK) activity levels (Hotfiel et al., 2018). DOMS typically occurs between 8 h and 24 h after the muscle demanding exercise, peak between 24 and 72 h and could last up to 7 days (Agostini et al., 2019). To this end, the exact cause of DOMS is unexplained, however, some scientists suggest that DOMS is not a single event of damage but series of various biochemical changes. It is believed that factors inducing DOMS include lactic acid, connective tissue damage, muscle temperature, muscle spasm, free radicals, inflammatory responses, nitric oxides, and enzyme efflux theory (Kim and Lee, 2014; Agostini et al., 2019). Furthermore, some authors suggested that pain is related to an adaptive remodelling of the myofibril proteins rather than myofibril damage (Agostini et al., 2019). Despite complexity of factors inducing occurring of DOMS, it is generally accepted that DOMS is caused by exercise-induced muscle damage (EIMD). However, recently DOMS has been associated with nerve growth factor (NGF) (Kim and Lee, 2014). Regarding the diagnosis of DOMS, no clear-cut definition has yet been proposed since there are shifting overlaps between muscle overload, damage, and injury (Hotfiel et al., 2018).

Various preventative and therapeutic strategies have been proposed to reduce symptoms of muscle soreness (Kim and Lee, 2014). The adequate management of DOMS is of most importance to elite athletes who are involved in multiday events since they have to maintain high level of performance despite of sustained strenuous effort (Agostini et al., 2019). Akin to EIMD, one of the most studied interventions includes nutrition (Heiss et al., 2019).

2.2.2. EIMD/DOMS and nutritional interventions

Most of nutritional interventions are based on potential of specific nutrient or supplement to induce anti-inflammatory response or on its antioxidant capacity. Most research of nutrients or supplements include caffeine, omega-3 fatty acids, taurine, vitamins C, D, and E, BCAA, glutamine, selenium, glutathione, coenzyme Q 10, creatine monohydrate, and various phytochemicals (Nieman et al., 2010; Kim and Lee, 2014; Heiss et al., 2019; Owens et al., 2018). Polyphenols with high antioxidant properties or some other antioxidant agents are suggested to be beneficial due to their ability to modulate inflammation in skeletal muscle. Beside inflammation, oxidative stress has been proposed to explain the way of initiating damage to skeletal muscle fibres following demanding exercise. However, caution is needed while supplementing with antioxidants because several studies indicated that reactive oxygen species (ROS) activate important cell signalling pathways which lead to skeletal muscle adaptations to training stimulus (e.g. mitochondrial biogenesis, the induction of the endogenous antioxidant defence, and hypertrophy). In other words, some degree of localised oxidation in the body is necessary (Halliwell, 2000). Hence, long-term use of nutritional recovery strategies, especially high doses of antioxidants on a regular basis, could hamper muscle adaptations to exercise training (Gomez-Cabrera et al., 2016; Owens et al., 2018).

It has been established that a healthy, balanced diet which an abundance of fruits and vegetables is required for athletes. However, there is justification to supplement with additional foods or specific nutrients since on a pro-level training and competition stress could be extremely high and recovery improbable before the next strenuous training session or competition. In this case supplementation could reduce negative effects of exercise damage, but as mentioned before, certain caution is necessary (Gomez-Cabrera et al., 2016; Owens et al., 2018). If the main goal is maximising adaptive response to a training stimulus then certain amount of inflammation is required. On the other hand, too much of it for an extended period of time can impair muscle function for long and disenable recovery form EIMD in time for the rest of training or competition programme (Owens et al., 2018). As stated before, the protective effect of diet and protective effect of antioxidants in the diet are not equivalent (Halliwell, 2000).

It is important to find the balance between adaptation and recovery which could be achieved with an individualised, periodized nutritional approach. Since we are still lacking the exact knowledge and understanding of functional foods mechanisms more studies are needed. Understanding the complex aetiology of EIMD and exert mechanisms of functional foods will allow more efficient implementation of nutritional interventions in maximising muscle adaptations, acceleration of muscle function recovery, and avoidance of performance impairments (Nieman et al., 2010; Owens et al., 2018). Although overall food intake is important to maintain health and good performance, regarding recovery, optimal pre- and post-exercise nutritional intake is prerequisite to accelerate muscle structure repair and muscle function recovery (Sousa et al., 2014). More research is needed do define better nutritional recommendations and optimal supplements dosing regimens (Nieman et al., 2010).

In the meantime, the most effective way to increase antioxidant intake is to consume a varied diet which abounds with foods that are naturally good sources of antioxidants such as fruit, vegetables, and whole grains. This strategy could help minimising the risk of exceeding intake. When there is a perceived need to supplement dietary intake, it seems reasonable to choose a product that provides a combination of antioxidants, at moderate levels, rather than large doses of a single nutrient.(Nieman et al., 2010). Though, if supplementation is needed, always should be remembered, that contamination by prohibited substances must be considered (Heiss et al., 2019).

2.2.2.1. Protein and amino acids

Dietary protein intake is a crucial part of the muscle protein turnover regulation, especially as a response to exercise (Owens et al., 2018). Moreover, it is generally accepted that a positive muscle protein balance is necessary to promote the muscle repair and adaptation from EIMD (Sousa et al., 2014). It has been stated that protein ingestion around the time of exercise bout enhance adaptive processes in both endurance and resistance type of training (Owens et al., 2018). On the other hand, it is less clear if protein intake can mitigate muscle damage symptoms. Evidence are inconclusive regarding protein or free amino acid intake effect on alleviation of muscle damage markers and acceleration of muscle function recovery. Reasons lie behind the fact that muscle protein turnover adaptations develop over time and do not correspond the acute muscle damage changes (Owens et al., 2018). The majority of studies examining the role of only protein supplementation in preventing or alleviating symptoms of EIMD have used branched chain amino acids (BCAA) only. It has been stated that the ingestion of amino acids could be able to reduce muscle damage (Sousa et al., 2014). If proteins are combined with carbohydrates in amount of 0.8-1.2 g CHO/kg/h and 0.2-0.4 g P/kg/h, ideally in the early recovery period, with a minimum content of 20 g high-quality protein, regarding existing evidence, recovery following EIMD may be accelerated (Sousa et al., 2014). Proteins are the most common nutritional strategy to attempt to reduce the negative side effects of muscle recovery (Jackman et al., 2010). However, they are often ingested in the form of shakes as a beverage and not as a whole food approach. In either case, protein intake should always be sufficient regarding person's needs. Still, conclusions have yet to be drawn given the link between protein supplementation and recovery after EIMD. Future studies should aim to more homogeneity in the study design and selection of markers of muscle damage and recovery (Owens et al., 2018).

2.2.2.2. Functional foods

Food with identified potential to induce positive physiological effects in a human body became generally known under the phrase "functional food". These effects are usually related to preservation and improvement of human health, or even disease prevention. In sports, these foods have been recognised as part of solutions to reduce negative effects that occur after intensive physical activity. In other words, functional foods synergistic action with other forms of recovery strategies could promote better and faster recovery for athletes (Owens et al., 2018).

The key components of functional foods are phytochemicals, compounds found in plants with possible beneficial effect. One of the most researched are called polyphenols and could be found in tea, coffee, cocoa, nuts, fruits, and vegetables (Owens et al., 2018). Although some studies provided promising results, up to the present time no nutrition agent has been demonstrated to have ground-breaking effects in the prevention and manifestation of DOMS (Heiss et al., 2019).

One of the most promising functional foods interventions are tart Montmorency cherries (*Prunus cerasus*) which showed some positive effect in every intervention they were used, according to the published studies until 2018. Their use stimulated muscle function recovery and successfully reduced muscle soreness following EIMD (Owens et al., 2018). Another well researched food is pomegranate because of its polyphenol-rich nutritive profile. In particular, group of ellagitannins showed some potential in promoting recovery from EIMD. Pomegranate extract could improve muscle recovery, if taken in the days before and after the intense workout. Also, it could reduce muscle soreness perception, however, no other marker of inflammation or muscle damage differed between groups supplemented or not with pomegranate extract. Owens et al. (2018) suggested after reviewing literature that pomegranate may be useful and effective intervention in promoting recovery, both for recreational and well-trained athletes.

2.2.2.3. Flavonoids

Flavonoids are the largest and most researched group of polyphenols and could be divided into subgroups regarding chemical structure: flavonols, flavones, flavanones, flavanols, anthocyanidins and isoflavonoids. Their functions in plants include pigmenting, signalling and infection or injury defence. In the human diet their role is considered to be anti-inflammatory, anti-viral, antioxidant, anti-obesity and anti-carcinogenic, although these properties are studied mostly in vitro using high doses of purified extracts. Human studies are still producing inconclusive data. Depending on the diversity of diet, dietary intake ranges from 50 to 800 mg/day. Important sources include tea, citrus fruits and juices, beers and ales, wines, melon, berries, apples, bananas, and onions (Nieman et al., 2010). Although flavonoids are bioactive molecules, their bioactivity is affected by their insufficient absorption, extensive conjugation, and metabolic transformations. Additionally, it is important to mention that positive effects of plant foods are result of complex mixture of phytochemicals and their mutual interactions, rather than impact of one single component. Flavonoids act synergistically increasing each other's bioavailability and decreasing elimination by competitive inhibition (Nieman et al., 2010).

Two polyphenols, quercetin and catechin, along with their derivates, caught the attention of researchers as compounds with potential to reinforce recovery after the damage provoking exercise. However, strong supporting evidence are lacking, hence, their application as EIMD recovery strategy is unsubstantiated (Owens et al., 2018). Quercetin is a flavonol found in berries, grapes, tomatoes, and tea. Although it showed good bioavailability after consumption, it failed to diminish inflammation, oxidative stress, and muscle soreness or ameliorate muscle function (Owens et al., 2018). Quercetin is the most researched flavonoid regarding exercise related outcomes (Nieman et al., 2010). Catechin is found in tea, and even though is connected to enhancing recovery, the same is not strongly supported (Owens et al., 2018). Both quercetin and catechin have been found in pistachio nut (Ojeda-Amador et al., 2019).

Phytochemicals, such as flavonoids, exert effects in vitro, but in vivo and human studies, little or no preventative or therapeutic effect was confirmed. They could be helpful or beneficial for people with poor diet and low antioxidant status, but for people already consuming varied diet, additional intake has no further benefits (Halliwell, 2012).

2.2.3. Downhill running

Downhill running is a form of unusual, often unfamiliar, eccentric exercise. Together with resistance or prolonged training, intermittent activities, and high intensity exercise, downhill running is one type of exercise that regularly result in EIMD symptoms (Owens et al., 2018). Downhill running substantially increases the probability of overuse running injury. metabolic cost increases during downhill running at steep angles. Compared to level running, the normal impact force peaks were dramatically larger for downhill running and smaller for uphill running. The normal impact force peak data suggest that the probability for musculoskeletal injury increases during downhill running and decreases during uphill running (Gottschall and Kram, 2005). It was stated that tenderness following downhill running is the greatest in the gluteus maximus, rectus femoris, vastus medialis, vastus lateralis, tibialis anterior, gastrocnemius, and biceps femoris. However, tenderness appears immediately rather than delayed. DOMS appears in the gluteal muscles, the quadriceps, and the anterior and posterior tibial muscles. Downhill running is not exclusively eccentric exercise, but is more functional activity, that way more reliable and closer to normal muscle reactions (Eston et al., 1995). With this in mind, downhill running during external valid conditions in sports, there are no isolated eccentric contractions that induce a “pure eccentric overload” as applied in numerous DOMS models. Instead, during sportive activities as running, change of directions, and jumps, eccentric contractions are shorter and part of the entire stretch-shortening cycle, also involving, and per time more, concentric contractions (Hotfiel et al., 2018).

3. EXPERIMENTAL PART

3.1. MATERIALS AND METHODS

3.1.1. Experimental design

Twenty-four participants, ages 18-50 years, were recruited for this randomized parallel study. Participants visited Sport and Exercise Physiology Laboratory on six different occasions. Each visit, except visit no. 2, was conducted in the morning, and was preceded by an overnight fast. On their first visit, participants were provided with a written and verbal brief on the requirements of the study and offered the opportunity to ask questions before providing written informed consent. After signing their consent form, they filled in a health questionnaire, and were subjected to their first screening measurements. Body mass and height were measured. Participants were then asked to perform the VO_2 max running test protocol which was in details explained to them. VO_2 max testing was followed by one-week recovery period, after which participants who were randomised to pistachio groups came back to the laboratory to collect their 2 weeks supplementation doses of pistachios. Following the two weeks supplementation period, participants came back to the laboratory early in the morning in the overnight fasted state. They were asked to abstain from intense exercise 48 hours prior to the experimental session and throughout data collection. Body mass, height and skinfolds were measured. Participants evaluated their feeling of muscle soreness using the Visual Analogue Scale (VAS). Muscle performance was assessed performing vertical jump (VJ) using the Takei® Jump Meter, and muscle function via Biodex machine, measuring Maximal Voluntary Contraction (MVC) for the leg extensors and flexors. Following listed tests, downhill run was performed as a muscle damage exercise bout. With completion of running, participants consumed their prescribed dose of pistachio and water, or water alone. Water was consumed *ad libitum* both during and after the running protocol. Participants came back to the laboratory for three consecutive days following the downhill run, and repeated VAS, VJ and MVC tests. At 24 hours and 48 hours post downhill run they consumed pistachios after completion of all the tests. On their last visit, 72 hours post downhill run, they did not consume pistachios. Scheme of the experimental design is presented in Table 1.

Ethics approval was granted by NHS Invasive/Clinical Research ethics committee (NICR).

Table 1. Study design

	Visit 1	**Visit 2***	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**
		SUPPLEMENTATION 2 weeks	BASELINE	24 h post MD	48 h post MD	72 h post MD
Consent form / Questionnaire	✓					
Body mass and height measurements	✓		✓			
VO_2 max testing protocol	✓					
Skinfold measurements			✓			
VAS			✓	✓	✓	✓
VJ			✓	✓	✓	✓
MVC			✓	✓	✓	✓
MD protocol			✓			
Pistachio/water		✓	✓	✓	✓	

VAS – Visual Analogue Scale, VJ – Vertical Jump, MVC – Maximal Voluntary Contraction, MD – Muscle Damage, *was not conducted early in the morning

3.1.2. Participants

Twenty-four male sport active participants were recruited to participate in the study. Participant characteristics are shown in Table 1. Eligible participants were well trained endurance athletes who train at least 5 hours per week. They had to be healthy (with no known metabolic diseases or eating disorders) and must not use supplements known to impact antioxidant or inflammatory status within 1 month of participation. Other exclusion criteria included smoking, musculoskeletal limitations and use of anti-inflammatory medications. Three participants volunteered for all three conditions, an additional twelve volunteered for two conditions while further nine participants volunteered for one condition. Participants were treated as independent samples between each condition.

Table 2. Participants characteristics

Participants	**Age (years)**	**Height* (cm)**	**Weight** (kg)**	**BMI* (kg/m²)**	**BF*** (%)**	**VO_2 max**	**Target HR**	**Dominant leg**
n=24	27±3.4	180,4±6,4	74,2±11,6	22,38±3,4	11,98±0,86	49,5±10,8	157±12	R n=20 L n=4

Data presented as Mean ±SD, BMI – body mass index, BF – body fat, target HR – target heart rate, 70% of maximal heart rate determined while performing VO_2 max test; *n=18, **n=21, ***n=17

3.1.3. Equipment

3.1.3.1. Treadmill

To complete all running protocols (VO_2 max test and downhill run), H/P/COSMOS PULSAR® treadmill was used. While performing either of the running protocols participants were secured with a chest belt attached to the safety arch of the treadmill. Protocols, as well as settings of the treadmill, are described in detail below.

3.1.3.2. Anthropometry measuring devices

Body weight was measured with SECA body weight scale in a fasted state. Height was measured with a digital wall-mounted stadiometer. Both of this measurement participants performed barefoot in their underwear. Skinfolds were measured with calliper by trained ISAK certificated personnel. Skinfolds measurements were taken on 8 different body sites (biceps, triceps, subscapular, iliac crest, supraspinal, abdominal, thigh and calf) and repeated twice for each site. If values varied more than 10%, measurement was repeated the third time. Average value was then calculated for each site.

3.1.3.3. Vertical Jump Meter

The Takei® Vertical Jump Meter was used as a measurement system to measure vertical jump height. This jump meter measures how high someone can perform a vertical jump from a standing position. It includes an adjustable waist belt connected to the rubber mat by a measurement cord (Figure 2.). It is a portable system, easy to store and user-friendly. Person stands on the rubber mat and set the belt around their waist. The strap should be rewind until strained/tense and meter set to zero (Figure 3.). After performing a maximal jump, jump height value (in cm) was recorded from an easy-to-read display (Cartwright Fitness, 2020).

Figure 2. The Takei® Vertical Jump Meter (source: habdirect.co.uk)

Figure 3. The Takei® Vertical Jump Meter (source: bishopshop.co.uk)

3.1.3.4. Biodex isokinetic dynamometer

The Biodex machine is a multi-mode computerized robotic measuring instrument designed to measure muscle strength (Figure 4.). The machine applies constant resistance against muscles in repetitive motions quickly and as powerfully as possible. While performing the test at the knee joint, the patient sits in an upright chair with his/her leg strapped to a movable part of the machine. The patient is instructed to fully straighten and then fully bend the knee for multiple repetitions depending on specific test. When performing these two motions, knee extension and knee flexion, the machine is able to measure the strength output by extensor and flexor muscles of the upper leg. Resistance varies throughout the test but is always at a safe resistance level to limit joint compression forces, allowing pain free motion, and producing quality data to measure strength output during the entire range of motion. This machine is often used as part of preventative or rehabilitation training process, or as screening tool (BIODEX, 2020).

Figure 4. Biodex isokinetic dynamometer (Biodex, 2020)

3.1.4. Physical performance tests

3.1.4.1. VO_2 max test

On their first visit, after signing the consent form, participants undergo a VO_2 max test to measure their maximal aerobic capacity. Prior to performing the test, participant’s code and anthropometric data were inputted into OMNIA software of the COSMED setup equipment. Participants wore a chest strap heart rate monitor during the whole time. They were secured with the chest belt attached to the safety arch of the treadmill. A face mask was placed on them and connected by a gas flow tube to the QUARK Cardio Pulmonary Exercise Test (CPET) computer. Gas analyses of the participants breath was taken throughout the performance to measure their maximal oxygen consumption. Participants first warmed up for 3 minutes at their own pace. Following the warm-up, VO_2 max test began at a speed of 5.6 mph (9 kmph) and 1% elevation of treadmill. Every minute, speed was increased by 1.2 mph (2 kmph) up to 9.9 mph (15.9 kmph). Once the last speed stage was reached, speed remained the same and only the treadmill elevation was increased by 1% every minute. Increase continued until exhaustion. Participants were instructed to step to the sides of the treadmill the moment they reach they limit. The peak heart rate was recorded and VO_2 max value was measured.

3.1.4.2. Muscle soreness

Muscle soreness level was assessed using Visual Analogue Scale (VAS). Participants rated their perceived pain level for five muscles or muscle groups and two positions of both legs: *m. quadriceps femoris, m. tibialis anterior, m. gastrocnemius, gluteus,* and *hamstrings* and at extended (0°) and flexed knee position (90°). Soreness level was marked on the 100 mm rating scale where 0 presented “no pain” and 100 “unbearable pain” depending on the discomfort. The distance of participants’ mark from the first anchor point (0 mm) was used to present muscle soreness level.

3.1.4.3. Muscle function

An isokinetic maximum voluntary contraction (MVC) was used to examine the muscle function of the knee flexors and extensors. Participants sat in a dynamometer chair and had their torso, hips and working leg securely strapped to the machine (Picture 3.). The working leg was fastened to the dynamometer lever 1 centimetre higher than the lateral ankle joint, while the lever hinge was aligned exactly next to the knee (lateral femoral condyle). The range of motion was set to amount of 90 degrees with the start at flexed position (90°) and stop at extended position (0°) at the knee joint. The MVC protocol was performed for two sets with three repetitions each for both legs. First set was performed at 60°/second (Nm^{-1}) and the other one at 120°/second (Nm^{-1}). Flexion and extension peak torque values were recorded.

3.1.4.4. Muscle performance

Muscle performance was assessed via measuring vertical jump height. Jump height was measured using Takei® jump meter described above. Participants performed three maximum jumps with 30 seconds rest in between. The hight of each jump (in cm) was displayed on the screen and recorded. The highest jump was considered while analysing the results.

3.1.5. Muscle damage protocol

Following completion of physical performance tests on their third visit, participants performed downhill run as a muscle damage protocol. For the purposes of this protocol, the treadmill was elevated to 10% and the treadmill's belt direction was reversed. As a result, conditions for running downhill at -10% decline were created. Participants were secured with the chest belt connected to the arch of the treadmill. Heart rate was recorded using POLAR® chest strap. They started the protocol with 5 minutes warm-up at their own pace. Once participants warmed up, the treadmill speed was adjusted to induce target heart rate which was set to 70% of participant's maximal heart rate determined by the VO_2 max test. Run lasted 40 minutes during which every five minutes participants were asked to estimate intensity using Rated Perceived Exertion (RPE) Borg Scale and heart rate was recorded. Treadmill speed was adjusted if needed to maintain convenient heart rate. After completion of 40 minutes run, participants cooled down for 5 minutes at their own pace.

3.1.6. Treatments and dietary control

Participants were randomized to one of three groups. First group (A) was control one, without any intervention to participants' usual diet and workout programme. Second group (B) was assigned to 42.5 g of pistachio added to their diet for 2 weeks prior to the muscle damage protocol. They also continued to supplement their assigned dose of pistachio following downhill run and two consecutive days afterwards. Third group (C) had lager dose of pistachio (85 g) added to their usual daily intake for two weeks prior the testing and for three days (testing and two afterwards). As already mentioned, some participants volunteered for more than one condition.

Figure 5. Pistachio packages

Shelled Californian pistachios were provided by American Pistachios Growers. They were weighed on a digital scale and packed in small plastic bags, one for each day (Figure 5.). Following the preliminary test visit and one-week recovery period, participants assigned to both pistachio groups came back to the lab to collect their two-weeks pistachio nuts supplies.

3.1.7. Statistical analyses

Statistical analysis was conducted using GraphPad Prism version 8.4.3. for Windows (GraphPad Software LLC, San Diego, California USA) and Microsoft Excel 365 (Microsoft Office Excel 365, 2019). Two-way ANOVA and mixed-design repeated measurements ANOVA were used to estimate the effect of pistachio supplementation and pistachio dose on post-exercise recovery. Dunnett's test was used for post-hoc analyses. Level of significance, P value, was set at 0.05.

4. RESULTS AND DISCUSSION

The main purpose of the present study was to investigate the effect of two weeks pistachio supplementation on muscle recovery following downhill running. Downhill running was chosen to be muscle damaging exercise bout, as previously reported elsewhere (Owens et al., 2018). Muscle recovery was assessed through physical performance tests, including VAS to determine perceived rate of muscle soreness, MVC to measure muscle function, and VJ to check for muscle performance. It was hypothesized that pistachio intake may promote muscle recovery from vigorous eccentric exercise, with addition that possible positive effect would be dose dependent.

There are numerous studies dealing with benefits of regular pistachio intake on overall health, weight management in overweight people, prevention of chronic noncommunicable diseases in healthy persons, and treatment of them in persons who already developed some of these conditions (Bulló et al., 2015; Terzo et al., 2017). At the same time, even though pistachios appear to possess characteristics suggested to positively affect post-exercise muscle recovery process, not many studies were conducted to confirm the stated. Preliminary studies investigating pistachio intake on performance and post-exercise recovery show opposing results. Nieman et al. (2014) hypothesized that 3 ounces (85 g) of pistachios per day for two weeks and on the day of 75-km long intense cycling would promote substrate utilization and improve performance compared to water only. Moreover, they expected attenuated inflammation, oxidative stress, and immune dysfunction during 21 h recovery period. On the contrary to their expectations, pistachio intake impaired performance and led to increased plasma levels of oxidative stress and inflammation markers. They revealed post-exercise presence of raffinose, sucrose, myo-inositol, and leukotoxin diol 9,10-DiHOME in the participants' blood during participation in the intervention group. These findings were attributed to the increased gut-permeability (Nieman et al., 2014). On the other hand, Celik et al. (2019) reported preventative effects of dietary pistachio supplementation on oxidative stress caused by strenuous soccer training program in young soccer players. The effect was manifested through maintenance of thiol/disulphide homeostasis (TDH). TDH was suggested to be an indicator of oxidative stress, and important part of antioxidant defence system, impaired after the strenuous training program (Celik et al., 2019).

Nuts in general are not fully explored in the context of their effect on exercise performance or muscle recovery which gives a plenty of room for research. Evidence suggest their positive effects on overall health, prevention, and treatment of chronic diseases, as well as weight management (Dreher, 2012). They should be taken in moderation because of their high energy value, but with their frequent implementation to the everyday diet these beneficial effects could be achieved.

4.1. MUSCLE SORENESS

Muscle soreness level was assessed through Visual Analogue Scale (VAS), a popular tool for the measurement of pain. Justification for its use, and proper way of analysing the results are described elsewhere (Dexter and Chestnut, 1995; Heller et al., 2016).

Perceived muscle soreness pain values are shown in Tables 3. and 4. for right and left leg, respectively. Graphic presentation of data could be found in Supplement in Figures 8-21.

As expected, perceived muscle soreness level increased statistically 24 h post downhill running. Effects retained until 48 h, and in most cases at 72 h timepoint experienced a decrease, with such trend suggesting appearance of DOMS. No statistical differences were observed between right and left leg regarding muscle soreness. Moreover, no intergroup differences were observed regarding muscle groups or positions, for any time point. There were significant ($p<0,0001$) time effects for flexion and extension, and significant ($p<0,0001$) time and subject effects for all muscles or muscle groups. The absence of the Time x Condition effect ($p>0.05$) implies that muscle soreness is not impacted by pistachio intake.

On the other hand, some intragroup differences were observed. Perceived muscle soreness level was statistically different in all groups, for both legs, for all muscle parameters in 24 h and 48 h timepoint compared to baseline, except for extension group A at 24 h timepoint, and gastrocnemius group B at 48 h for right leg. At 72 h, quadriceps, hamstrings, gluteus, gastrocnemius, and extension in group C, and gastrocnemius in group A for right leg kept statistically significant differences. Regarding left leg quadriceps A, B, and C, hamstrings B and C, gluteus C, tibialis anterior C, and extension A were still statistically different at 72 h timepoint. Although cannot be concluded considering lack of statistical significance, trend could be noticed that muscle soreness for both legs is prolonged in the group C, the one assigned to 85 g of pistachio consumption.

Table 3. VAS muscle soreness score for right leg

Muscle group/position	Group	Baseline	24 h	48 h	72 h
Quadriceps m.*	A	4.6 ± 4.7	35.1 ± 19.6[c]	34.0 ± 22.8[b]	15.9 ± 16.1
	B	4.9 ± 4.8	29.5 ± 20.1[b]	35.2 ± 15.5[d]	20.9 ± 21.2
	C	3.9 ± 6.0	22.0 ± 15.0[c]	33.7 ± 22.1[c]	15.8 ± 11.2[b]
Hamstrings*	A	7.4 ± 10.2	28.7 ± 21.5[b]	31.2 ± 20.4[b]	14.6 ± 16.2
	B	5.3 ± 5.3	22.8 ± 18.2[b]	29.0 ± 17.3[c]	17.0 ± 21.3
	C	2.9 ± 3.9	21.1 ± 13.8[c]	28.7 ± 21.6[b]	13.6 ± 11.5[b]
Gluteus*	A	5.2 ± 5.1	29.8 ± 15.6[c]	29.7 ± 19.1[b]	13.9 ± 13.5
	B	4.8 ± 5.4	30.0 ± 26.0[b]	25.7 ± 15.3[c]	15.2 ± 19.1
	C	2.0 ± 2.9	22.6 ± 12.4[d]	23.9 ± 17.8[b]	8.9 ± 7.8[a]
Gastrocnemius m.*	A	4.8 ± 4.2	27.4 ± 22.7[b]	29.5 ± 24.3[b]	16.8 ± 16.3[a]
	B	8.6 ± 13.1	23.7 ± 20.2[a]	23.6 ± 26.1	21.6 ± 28.1
	C	3.9 ± 4.8	23.2 ± 23.4[a]	30.4 ± 28.8[b]	15.1 ± 15.2[a]
Tibialis anterior m.*	A	3.9 ± 4.2	14.9 ± 9.4[c]	23.8 ± 20.5[a]	10.8 ± 9.6
	B	3.9 ± 3.2	22.4 ± 17.5[b]	18.6 ± 19.5[a]	13.9 ± 20.8
	C	2.9 ± 5.3	15.7 ± 13.3[a]	24.1 ± 21.1[a]	9.9 ± 8.5
Extension*t	A	5.9 ± 7.9	17.8 ± 18.1	26.8 ± 24.4[a]	17.2 ± 15.1
	B	5.5 ± 5.6	23.9 ± 16.4[b]	25.4 ± 14.9[b]	15.9 ± 19.6
	C	4.6 ± 6.6	16.6 ± 9.5[b]	29.7 ± 18.7[c]	14.4 ± 13.1[a]
Flexion*t	A	4.8 ± 6.8	22.0 ± 15.7[b]	23.8 ± 19.2[b]	12.4 ± 12.3
	B	5.8 ± 11.5	20.9 ± 16.8[a]	21.3 ± 16.9[a]	12.4 ± 18.0
	C	7.0 ± 6.8	17.9 ± 11.1[b]	25.7 ± 17.2[b]	11.4 ± 10.5

Values are expressed as mean ± SD. Group A: control group (no diet interventions), group B: 42.5 g pistachio, group C: 85 g pistachio.
[*] p<0,0001, significant time and subject effects
[*t] p<0,0001, significant time effects
[a] p<0,05, significantly different from baseline for its own group
[b] p<0,002, significantly different from baseline for its own group
[c] p<0,0002, significantly different from baseline for its own group
[d] p<0,0001, significantly different from baseline for its own group

Table 4. VAS muscle soreness score for left leg

Muscle group/position	Group	Baseline	24 h	48 h	72 h
Quadriceps m.*	A	5.2 ± 5.5	36.4 ± 21.6[c]	32.9 ± 22.6[b]	16.4 ± 14.3[a]
	B	4.6 ± 5.8	27.7 ± 20.4[b]	31.9 ± 13.2[d]	17.7 ± 18.8[a]
	C	3.6 ± 5.3	20.3 ± 11.8[c]	29.3 ± 22.9[b]	14.2 ± 11.1[a]
Hamstrings*	A	7.1 ± 11.3	24.9 ± 18.9[b]	26.7 ± 22.3[a]	16.7 ± 14.8
	B	4.0 ± 5.7	25.2 ± 15.9[c]	29.2 ± 16.2[c]	19.1 ± 20.7[a]
	C	3.5 ± 4.3	21.5 ± 16.5[b]	25.1 ± 22.4[b]	13.1 ± 15.6[a]
Gluteus*	A	6.4 ± 7.6	28.7 ± 15.7[b]	29.1 ± 19.2[b]	11.3 ± 10.0
	B	5.0 ± 6.0	27.4 ± 22.9[a]	24.1 ± 11.3[d]	12.6 ± 12.6
	C	1.9 ± 2.5	22.7 ± 14.5[c]	20.3 ± 16.9[b]	9.0 ± 7.9[b]
Gastrocnemius m.*	A	4.6 ± 4.5	27.4 ± 23.1[b]	29.4 ± 25.1[b]	17.0 ± 20.8
	B	6.6 ± 11.4	25.0 ± 21.0[b]	24.1 ± 25.1[a]	21.1 ± 25.6
	C	3.9 ± 5.9	19.5 ± 18.6[a]	25.1 ± 25.6[a]	13.8 ± 15.2
Tibialis anterior m.*	A	4.6 ± 4.9	13.4 ± 9.9[b]	25.0 ± 20.7[a]	11.1 ± 11.4
	B	3.6 ± 3.7	22.1 ± 18.3[b]	20.3 ± 19.2[a]	13.5 ± 19.6
	C	2.3 ± 3.0	12.4 ± 9.9[b]	19.0 ± 18.3[a]	9.7 ± 8.4[a]
Extension*t	A	4.6 ± 6.8	25.2 ± 17.3[b]	25.6 ± 21.5[a]	17.9 ± 17.0[a]
	B	6.4 ± 10.5	22.1 ± 16.6[a]	26.8 ± 15.9[b]	17.4 ± 21.7
	C	4.9 ± 6.9	19.3 ± 12.0[b]	29.3 ± 18.5[c]	11.6 ± 13.0
Flexion*t	A	4.5 ± 4.8	23.4 ± 16.7[b]	24.1 ± 23.1[a]	15.4 ± 18.2
	B	5.9 ± 10.0	20.4 ± 16.8[b]	24.6 ± 18.2[c]	16.0 ± 23.3
	C	6.4 ± 7.5	17.0 ± 11.7[c]	23.5 ± 19.1[a]	10.9 ± 12.9

Values are expressed as mean ± SD. Group A: control group (no diet interventions), group B: 42.5 g pistachio, group C: 85 g pistachio.

* p<0,0001, significant time and subject effects

*t p<0,0001, significant time effects

[a] p<0,05, significantly different from baseline for its own group

[b] p<0,002, significantly different from baseline for its own group

[c] p<0,0002, significantly different from baseline for its own group

[d] p<0,0001, significantly different from baseline for its own group

4.2. MUSCLE FUNCTION

Muscle function was assessed via measuring Maximal Voluntary Contraction (MVC) on an isokinetic dynamometer. Peak torque values for extension and flexion at two different speeds are presented in Tables 5. and 6. for right and left leg, respectively. Graphic presentation of data could be found in Supplement in Figures 22-29.

Contrary to expected, in most cases no differences were found in peak torque values for any timepoint compared to baseline. Only in left leg group C, 24 h timepoint was significantly decreased in comparison to baseline at both extension speeds. Additionally, in left leg group A 72 h timepoint odd decrease was observed. There were no statistical differences between legs or groups. Significant ($p<0,05$) time effects were found but only for both extensions of the left leg.

As shown in Tables 5. and 6., MVC data are quite disparate, that way leaving little space for drawing firm conclusions. These unexpected values could be explained by a non-existent familiarisation session. Since MVC was the most challenging of the three physical performance tests, it is possible that oral instructions were not enough, and that baseline experience due to familiarisation with equipment and task affected performance the following day (Ormsbee et al., 2015). According to MVC data, muscle damage did not occur, since loss of muscle strength is the best indirect marker of muscle damage (Owens et al., 2018), which was in contrast with VAS data.

Table 5. MVC peak torque for right leg

MVC	Group	Baseline	24 h	48 h	72 h
Extension 60°/sec (Nm^{-1})	A	172.6 ± 65.7	158.2 ± 59.8	170.3 ± 51.1	176.0 ± 50.1
	B	169.4 ± 42.3	173.3 ± 42.2	172.6 ± 38.9	179.7 ± 47.9
	C	177.9 ± 39.3	164.9 ± 41.0	167.8 ± 45.7	179.2 ± 46.0
Flexion 60°/sec (Nm^{-1})	A	82.7 ± 33.3	80.0 ± 36.6	86.3 ± 33.7	86.9 ± 28.1
	B	87.7 ± 35.0	94.1 ± 23.6	92.4 ± 21.6	94.2 ± 26.5
	C	84.6 ± 22.5	83.3 ± 24.1	91.0 ± 25.2	92.9 ± 28.5

Extension 120°/sec (Nm^{-1})	A	147.4 ± 46.2	141.2 ± 44.8	146.5 ± 35.9	150.8 ± 42.0
	B	148.6 ± 40.3	151.8 ± 44.2	152.7 ± 36.2	152.4 ± 39.4
	C	146.3 ± 37.4	139.9 ± 35.9	146.7 ± 39.6	148.5 ± 37.0
Flexion 120°/sec (Nm^{-1})	A	75.6 ± 26.5	69.9 ± 28.4	72.6 ± 30.1	77.0 ± 28.6
	B	79.1 ± 31.0	83.0 ± 22.6	82.1 ± 28.6	85.5 ± 27.8
	C	76.3 ± 19.7	76.1 ± 22.8	78.7 ± 26.2	80.6 ± 27.8

Values are expressed as mean ± SD. Group A: control group (no diet interventions), group B: 42.5 g pistachio, group C: 85 g pistachio.

Table 6. MVC peak torque for left leg

MVC	Group	Baseline	24 h	48 h	72 h
Extension^{*t} 60°/sec (Nm^{-1})	A	158.4 ± 43.6	137.4 ± 40.5	166.2 ± 51.5	139.1 ± 40.2*
	B	170.1 ± 43.5	153.6 ± 36.5	160.8 ± 45.2	161.8 ± 47.9
	C	178.7 ± 42.6	159.4 ± 42.4*	172.1 ± 44.7	170.8 ± 52.1
Flexion 60°/sec (Nm^{-1})	A	81.4 ± 40.3	80.8 ± 31.8	78.8 ± 35.0	79.6 ± 34.8
	B	81.6 ± 38.5	90.5 ± 24.7	82.8 ± 23.9	88.0 ± 28.5
	C	84.3 ± 23.6	79.5 ± 19.1	86.8 ± 25.8	92.9 ± 27.0
Extension^{*t} 120°/sec (Nm^{-1})	A	143.9 ± 48.0	137.4 ± 40.5	139.1 ± 40.2	143.0 ± 40.4
	B	144.3 ± 40.9	130.4 ± 35.3	137.0 ± 38.2	139.0 ± 42.1
	C	145.7 ± 36.1	130.9 ± 39.9*	147.4 ± 42.5	144.4 ± 42.2
Flexion 120°/sec (Nm^{-1})	A	73.7 ± 31.7	70.0 ± 26.6	67.5 ± 28.6	71.4 ± 30.6
	B	75.4 ± 35.9	76.6 ± 26.6	75.0 ± 27.5	81.5 ± 32.7
	C	74.9 ± 22.3	73.3 ± 25.5	73.9 ± 28.3	79.7 ± 28.3

Values are expressed as mean ± SD. Group A: control group (no diet interventions), group B: 42.5 g pistachio, group C: 85 g pistachio.
^{*t} p<0,05, significant time effects
* p<0,05, significantly different from baseline for its own group

4.3. MUSCLE PERFORMANCE

Muscle performance was checked by performing vertical jump (VJ).

Maximal VJ height in cm and percentage of change of maximal VJ are presented in Figures 6. and 7., respectively. Baseline maximal VJ height values were 53 ± 9.2, 53 ± 9.5 and 54 ± 7.6

cm in groups A, B and C, respectively. In all three groups, decrease in jump height could be observed 24 h post vigorous exercise bout, although only in group C that decrease was statistically significant. In group B decrease continued 48 h post workout, whereas all three groups met an increase at 72 h timepoint. Considering percentage of change, group C failed to return to pre-exercise values, while group B even exceeds theirs, however non significantly. 24 h decreases were -4.4% in group A, -2.8% in group B and -4.9% in group C. 72 h post workout, group A, B and C jumped 0.4%, 1.9% and -0.4%, respectively, of their baseline maximal jumps. No differences were observed between groups either. However, significant time and subject effects were found.

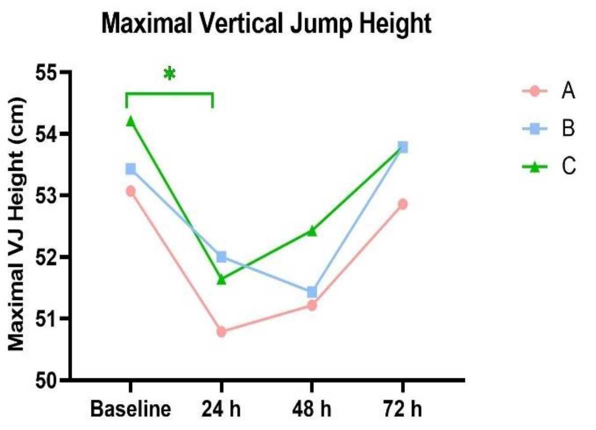

Figure 6. Maximal vertical jump height in cm

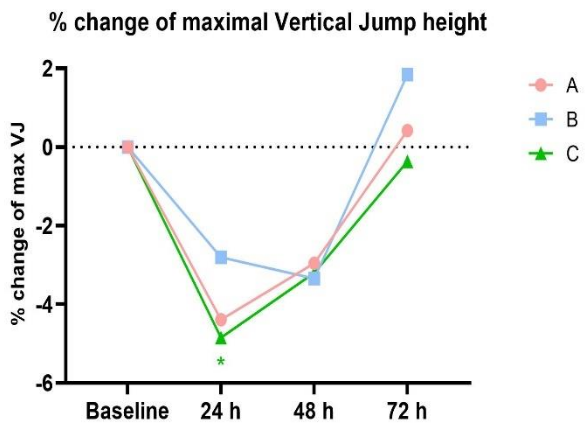

Figure 7. Percentage change of maximal vertical jump height

The downhill running as a muscle damage protocol successfully elicited an increase in muscle soreness reaching its peak at 24-48 h post-exercise similarly to Miller at al. (2004) and Ormsbee et al. (2015). Despite inducing DOMS, decrease in muscle force production was not observed, contrary to some previously reported evidence (Herrlinger et al., 2015). However, indirect markers of muscle damage are being reviewed since they could be limited in terms of their sensitivity and bias. Some authors argue that changes following eccentric exercise provide evidence for myofibrillar remodelling and adaptation, rather than muscle damage or inflammation. Proteomic is suggested for studying skeletal muscle adaptations to eccentric exercise (Ormsbee et al., 2015). To more precise determine of muscle damage, serum levels of skeletal muscle enzymes and proteins, such as creatine kinase (CK), lactate dehydrogenase, myoglobin, and troponin, should be quantified as direct and the most useful markers of muscle injury. Blood evaluation, in order to determine muscle damage, could also include various markers of inflammation, oxidative stress, muscle stress, and muscle catabolism (Herrlinger et al., 2015).

Participants who were involved in more than one trial, could have encounter repeated bout effect (RBE), which was shown as potential limitation to cross-over studies (Owens et al., 2018). The chance of RBE was minimised with long enough periods between trials, of 3-4 weeks.

Although inconclusive, lower dose of pistachio demonstrated a weak trend towards beneficial effect on recovery following muscle damage exercise bout. This is in accordance to some evidence claiming that lower doses of antioxidant or antioxidant plus other nutrients, may be more beneficial than the higher ones despite still mixed and confusing data (Halliwell, 2012).

More research is needed on the impact of pistachios on muscle recovery following strenuous, unfamiliar, eccentric exercise. Validation of these preliminary results is required on a bigger population and possibly with more controlled conditions. Study design could be improved by better familiarisation session, collecting blood samples, and checking for markers of muscle damage and inflammation. Implementation of monitoring of the diet, sleep quality, and training programme between trials could be out of great importance for more controlled study design.

5. CONCLUSIONS

- The occurrence of DOMS was consistent with literature, reaching its peak at 24-48 h post intense exercise bout, and diminished at 72 h, however, it was not confirmed to be affected by pistachio intake.
- The absence of significant muscle strength differences and disparate results could suggest the importance of familiarisation session prior to this type of test before drawing conclusions connected to pistachio intake.
- Smaller amount of pistachios, as antioxidants rich foods, appears to be more beneficial than the higher one in terms of drop in muscle performance and reaching the baseline value post intensive exercise bout.
- Pistachios have not been demonstrated as a good recovery food, nevertheless; higher amounts (85 g) are showing a trend towards a negative impact on muscle performance, and DOMS duration.
- Preliminary results require further validation with bigger population group and possible improvement of the study design through familiarisation session, collecting blood samples in order to check for muscle damage, oxidative stress and inflammation markers, and tighter control of variables such as the diet, sleep quality and training programme.

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7. SUPPLEMENT

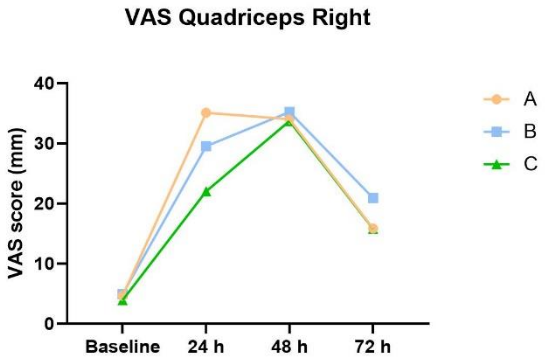


Figure 8. VAS Quadriceps Right leg

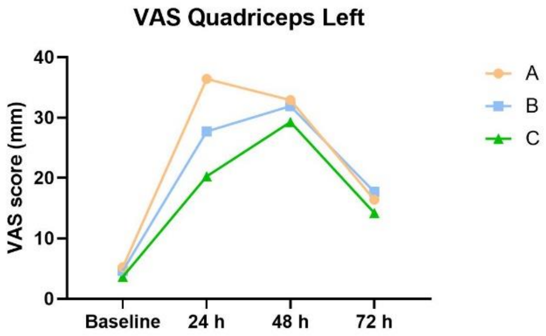


Figure 9. VAS Quadriceps Left leg

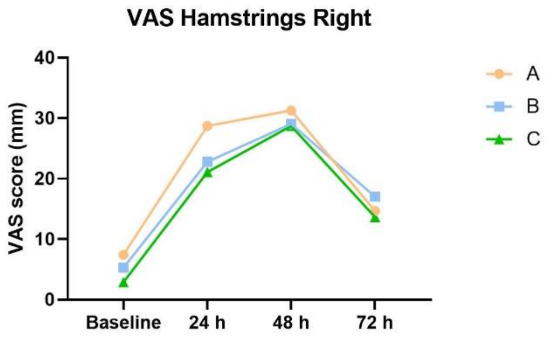


Figure 10. VAS Hamstrings Right leg

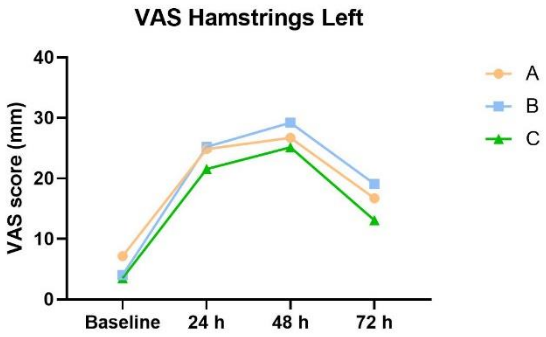


Figure 11. VAS Hamstrings Left leg

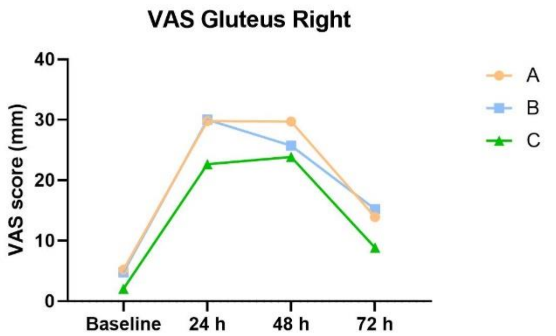


Figure 12. VAS Gluteus Right leg

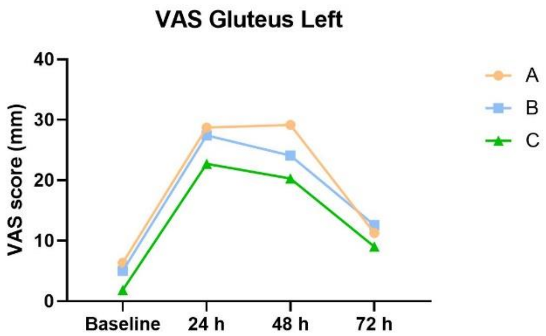
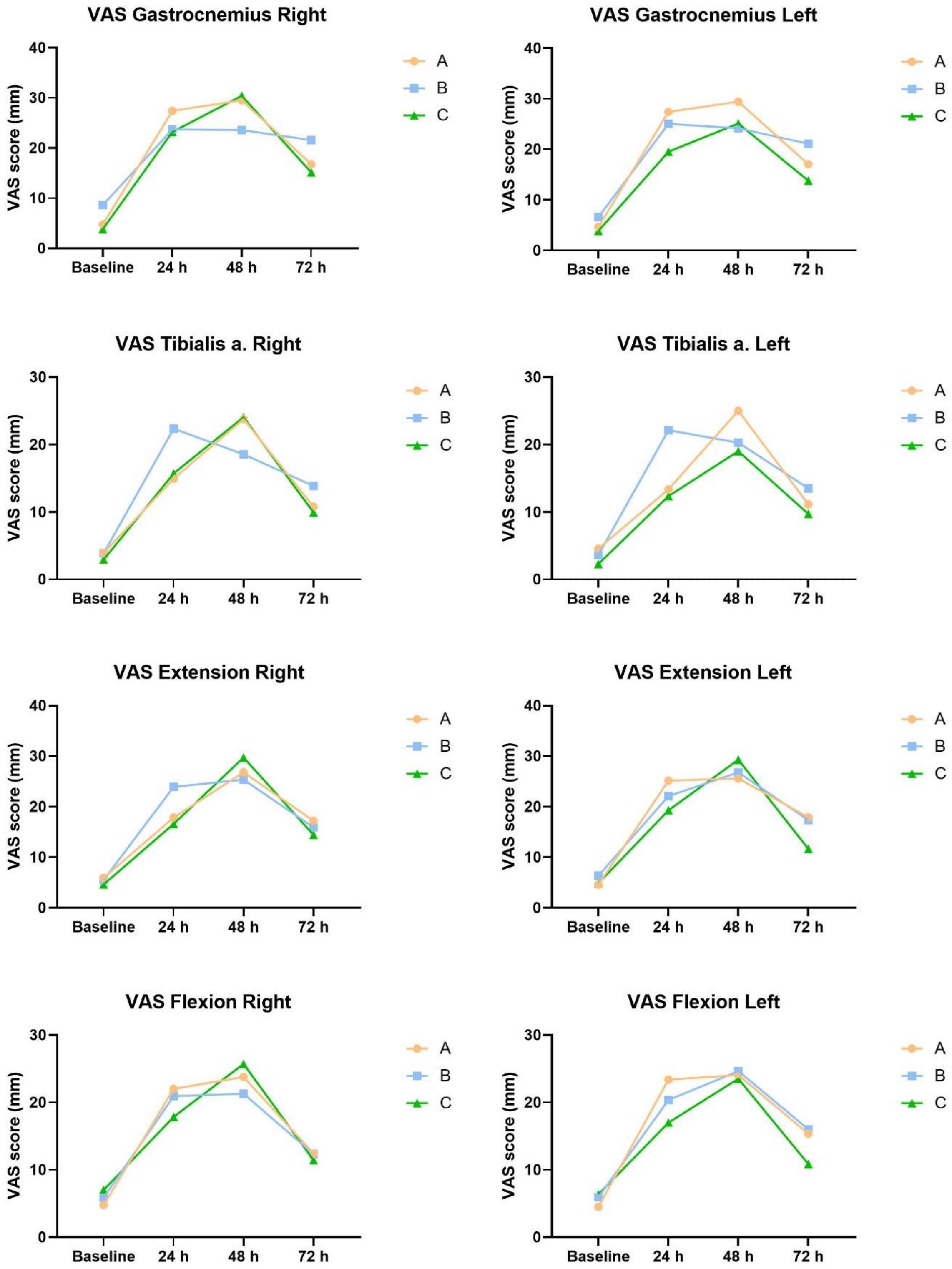


Figure 13. VAS Gluteus Left leg



Figures 14-21. VAS Gastrocnemius, Tibialis anterior, Extension and Flexion for Right and Left leg

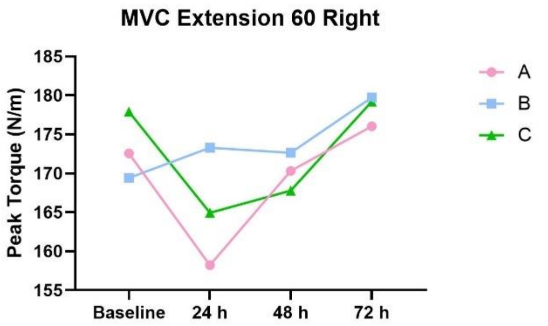


Figure 22. MVC Extension 60 Right leg

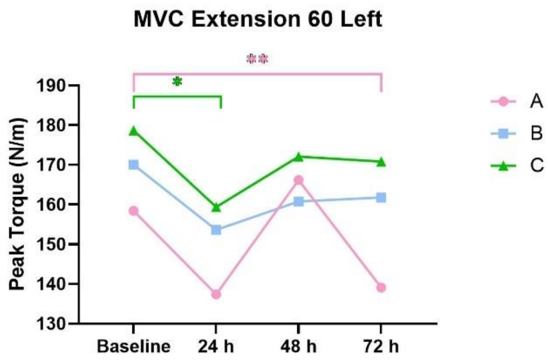


Figure 23. MVC Extension 60 Left leg

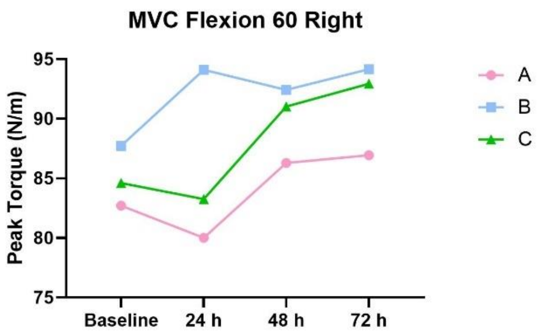


Figure 24. MVC Flexion 60 Right leg

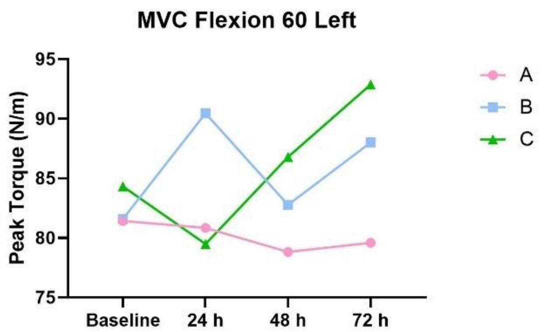
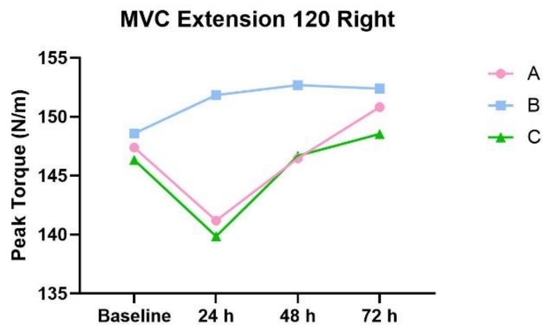


Figure 25. MVC Flexion 60 Left leg



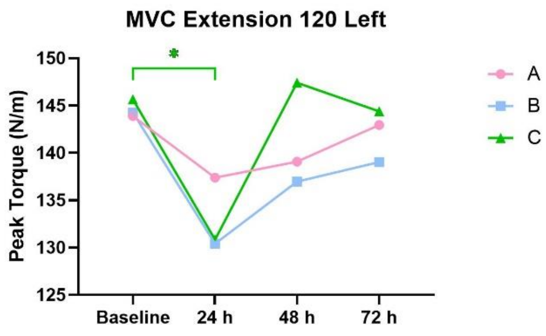
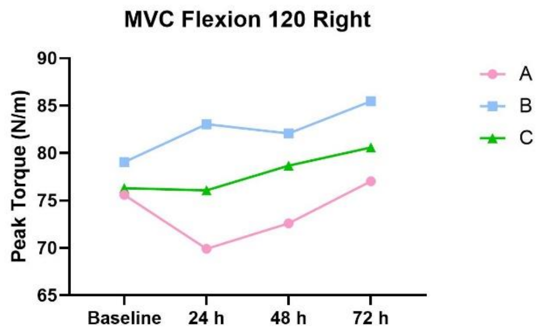


Figure 26. MVC Extension 120 Right

Figure 27. MVC Extension 120 Left leg



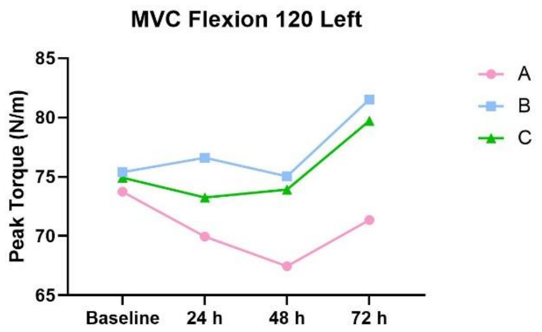


Figure 28. MVC Flexion 120 Right leg

Figure 29. MVC Flexion 120 Left leg

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.

Ivana Ovčina

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