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Polyphenols and Athletic Performance: A Review on Human Data

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Abstract

Exercise-induced aerobic bioenergetic reactions in mitochondria and cytosol increase production of reactive oxygen species. Many efforts have been carried out to identify dietary strategies or micronutrients able to prevent or at least attenuate the exercise-induced muscle damage and stress. A lot of studies are about how effective dietary intervention and oral antioxidant supplementation may be in reducing oxidative stress in athletes who exercise intensively. Commonly used nonenzymatic supplements have been proposed as ways to prevent exercise-induced oxidative stress and hence improve adaptation responses to endurance training. Plant-derived bioactive compounds can repress inflammation by inhibiting oxidative damage and interacting with the immune system. This review focuses on polyphenols and phytochemicals present in the plant kingdom that have been recently suggested to exert some positive effects on exercise-induced muscle damage and oxidative stress. This review will summarize some of the actual knowledge on polyphenolic compounds that have been demonstrated both to exert a significant effect in exercise-induced muscle damage and to play a biological/physiological role in improving physical performance. Overall, the pooled results show that polyphenols are viable supplements to improve performance in athletes.

Keywords: antioxidant, athletic performance, nutrition, polyphenols, oxidative stress, sport

1. Introduction

Many authors have noted that physical exercise induces an increase in production of free radicals and other reactive oxygen species (ROS) [1]. Current evidence indicates that ROS are the primary reason of exercise-induced disturbances in muscle redox balance, and it was observed that severe disturbances in redox balance have been shown to promote oxidative injury and muscle fatigue impairing the exercise performance [2, 3].

During the physical exercise practice, it is possible for the activation of some biological processes, such as the macrophages infiltration, the movement of electrons that occurs at the level of the transport chain on the mitochondrial ridges, the catabolism pathway of the purines, or the reaction catalyzed by the enzyme xanthine oxidase, which all may lead to the release of ROS. On the basis of the above-mentioned information, sportsmen have to improve their antioxidant defense systems to overcome the

exercise-induced oxidative damage. It is well established that the increase in reactive oxygen species and free radical production during exercise has both positive and negative physiological effects. In 2008, for the first time, moderate exercise has been defined as an antioxidant, explaining that the mild burst of ROS, generated by training, acts as a signal responsible for the activation of signaling pathways that lead to the induction of antioxidant enzymes in human tissue [4]. To prevent these hypothetically negative or side effects of physical exercise, supplementation with different types of antioxidants has been used in a great number of studies [2].

The term “antioxidant” in general indicates the molecules capable of preventing, delaying or, in some cases, completely canceling oxidative damage to specific target molecules. For example, the superoxide dismutase enzyme, the catalase enzyme, and the glutathione peroxidase enzyme are endogenous antioxidants; glutathione, vitamins E, C and A, and coenzyme Q10 (CoQ10) are nonenzymatic molecules with antioxidant properties [5].

The antioxidants can also be taken through the “exogenous antioxidant” diet, and this supplementation can improve the ability to protect muscle fiber, during training, from oxidative damage caused by fatigue. In fact, the deficiency of antioxidants could induce an increased predisposition to oxidative damage induced by exercise and therefore compromise the sporting performance [6].

Over the past few decades, many attempts have been made to improve antioxidant potential, and therefore increase physical performance by improving nutrition, training programmers, and other related factors. Recently, the problem of whether or not athletes should use antioxidant supplements is an important and highly debated topic.

In the context of this chapter, information in brief about the well-known and recently used antioxidants in particular the polyphenols is given. This review describes only human trials. The effects of these antioxidants on exercise performance and exercise-induced oxidative stress are also explained.

2. Oxidative stress and antioxidants

All aerobic organisms constantly synthesize free radicals as part of normal metabolic processes. Free radicals are chemical species with an unpaired electron in their outermost orbital; the free radicals that can be formed precisely by the oxygen molecules are indicated by the acronym ROS, that is, reactive oxygen species [7, 8].

Free radicals and reactive oxygen species are the main oxidizing agents in cellular systems and are involved in aging and the onset of many types of diseases. They are physiologically produced in different cellular biochemical reactions occurring in the body, such as in mitochondria for aerobic oxygen production, in fatty acid metabolism, in drug metabolism, and during activity of the immune system. On the other hand, free radicals can also be produced by exogenous factors such as pollution, bad lifestyle habits, UV rays, ionizing radiation, and psychophysical stress resulting from intense physical activity [9–11]. Although these free radicals have positive effects on immune reactions and cellular signaling, they are also known to have negative effects, such as oxidative damage of lipids, proteins, and nucleic acids. Organisms are equipped with antioxidant defense systems that protect cells from the toxic effects of free radicals [9–11].

Antioxidants are molecules able to give an electron to free radicals, neutralizing, diminishing, or eliminating their ability to damage cells and the main biomolecules such as nucleic acids, proteins, and lipids.

As already mentioned, it is possible to divide the antioxidants into two categories: enzymatic antioxidants, such as the enzyme superoxide dismutase (SOD), the

enzyme glutathione peroxidase, or the enzyme catalase; nonenzymatic antioxidants, such as glutathione, vitamin E, vitamin C, and bilirubin.

These “endogenous” antioxidants have the function of delaying or preventing the oxidation of extracellular and intracellular biomolecules. We know that even antioxidants taken from the diet, such as vitamins and minerals, can condition the oxidative state of the body. Some mammals, except humans, possess the biochemical mechanism that allows them to synthesize vitamin C. And this information can be useful for the purpose of supplementary integration during a sports performance [9].

Therefore, oxidative stress produces oxidative damage that can influence various physiological functions and can be defined as an imbalance between oxidants and antioxidants in favor of oxidants.

The production of ROS induced by exercise is an important signaling path for inducing biological adaptations to training, but ROS production could also have a deleterious impact on cells and tissues, that is, lipid and protein peroxidation. This concern has led some experts to suggest consuming more dietary supplements and supplements containing antioxidant to mitigate ROS production which can cause excessive oxidative stress during and after exercise [9–12].

2.1 Oxidative stress and physic activity

We have said that free radicals are normally generated during various physiological mechanisms. But their production increases considerably during a physical activity; in this situation, the skeletal muscles need a greater oxygen supply, resulting in an obvious change in the blood flow between the various organs. Subsequently, muscle damage induced by physical exercise causes infiltration of phagocytes (macrophages and neutrophils) in the area where the lesion occurred. All these physiological changes that occur during acute exercise cause an increase in the production of ROS, with consequent oxidative damage to the biomolecules. Through the use of biochemical and molecular techniques, it is now possible to evaluate events that occur at the cellular level and demonstrate in an increasingly precise manner, as free radicals certainly play a role in the physiological adaptations observed in the athlete after training. But free radicals generated by exercise can have both positive and negative physiological effects [10].

Exercise-induced oxidative stress associated with increased free radical production has been studied for 40 years, since it was first reported in 1978. In the study, subjects perform a 60-min cycle ergometer exercise at 50% VO_2 max intensity and reported increased levels of expired pentane, an index of lipid peroxidation [10]. Subsequently, in 1987, a study was carried out on six young men who performed an incremental load exercise on a cycle ergometer until it was exhausted, and it was discovered that the blood levels of reactive substances to thiobarbituric acid (TBARS), another marker of lipid peroxidation, increased [24]. In another study, in 1988, in which eight highly trained young men performed cycle ergometer exercise for 90 min at 65% VO_2 peak intensity, the levels of GSH, a nonenzymatic antioxidant, decreased, whereas the GSSG levels conversely increased [10].

The expression of the proteins involved in mitochondrial biogenesis, that is, the receptor gamma-activator receptor activated by the peroxisome alpha proliferator (PGC-1 α) is increased by regular resistance training. In fact, ROS stimulates the cascade of mitochondrial biogenesis precisely in response to endurance training, that is, the chronic muscular contractions [12]. The newly formed mitochondria are highly efficient and have the capacity to synthesize less ROS for the same amount of adenosine triphosphate product, that is, they are more functional.

For example, expression of PGC-1 α in skeletal muscle was significantly increased following 4 weeks of endurance training [13], indicating a skeletal muscle

contraction-stimulated mechanism of mitochondrial biogenesis. During muscle contraction, ROS can also be generated through mechanisms that do not involve the mitochondria. In fact, it has been shown that muscle contraction causes an increase in superoxide ion in the cytosol, before the increase in mitochondria occur. It has been hypothesized that the activity of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) causes the superoxide ion to increase in the cytosol [14]. Accordingly, ROS production (level of H_2O_2) was previously shown to increase in isolated mitochondria after acute muscle contraction in comparison with rested skeletal muscle biopsy sample [10].

ROS production leads to muscle fiber damage, which eventually results in muscle fatigue.

ROS production leads to damage to the muscle fiber, which then results in muscle strain. However, increasing evidence suggests that small stimuli, such as a low concentration of ROS, are able to stimulate the transcription of the major genes that encode proteins with antioxidant power. Superoxide dismutase (SOD) and glutathione are the examples of important molecules capable of defending cells from ROS-induced oxidative stress. Being able to deepen the mechanism of correlation between muscle fatigue and oxidative damage could be an important strategy for nutritional interventions aimed at increasing the performance of the exercise. An effective strategy could be antioxidant supplementation, considering the effects of scavenging ROS, which could lead to a decrease in muscle damage caused by prolonged exercise [14].

To date, there has been a plethora of reports on the effects of acute aerobic exercise on oxidative stress markers. Most of these studies have been conducted on young healthy men, and the difficulty of linking the various studies depends on the fact that, in each study, the training status is different. The most common exercises are those in which the athletes analyzed use a cycle ergometer or treadmill, in which the subjects, in an air-conditioned laboratory, generally engage in a maximal or submaximal exercise for 10–90 min.

Other studies have evaluated the effects of eccentric contraction exercises, such as downhill exercises. The most analyzed biological sample is blood. In a limited number of studies, skeletal muscle, exhaled air, and urine were also examined. Oxidation products of lipids, proteins, and DNA (i.e., MDA, PC, and 8-OHdG) have been used as oxidative stress markers; antioxidant levels and the redox balance in tissues were also assessed [3, 8].

Oxidative stress can be induced by aerobic exercise, but it can also be activated by anaerobic exercise. In fact, in addition to studies using sprint exercises, other studies have evaluated the effects of resistance exercises (formation of muscle groups throughout the body through the use of different types of resistance exercises) on oxidative stress markers. In a study in which 12 highly trained youths performed three sets of eight types of resistance exercise at 10 repetition loads, levels of malondialdehyde, a marker of lipid peroxidation, increased in the blood [8, 9]. This is one of the many tests that demonstrate that resistance exercises involving the whole musculature of the body modify the blood levels of oxidative stress markers [8]. But also local resistance exercises (defined as exercises that train a specific muscle group using a single type of resistance exercise) modify the oxidative stress markers of the blood [8]. While all these studies have assessed oxidative stress by measuring the change in blood parameters, other studies have observed muscle biopsies, to demonstrate that local resistance exercises increase oxidative stress in skeletal muscles [8].

On the other hand, other studies have shown that levels of oxidative stress in the blood are not conditioned by resistance exercises. Probably, the discrepancy of this data may have been conditioned by the training status. However, a study

of individual changes in oxidative stress responses to eccentric exercise demonstrated high inter-individual variability after exercise of eccentric knee extension, even in subjects with the same training status [8]. Furthermore, this study showed that in about one person in three exercise-induced oxidative stress was unexpected or negligible (response rate of 5% or less). These data reasonably suggest that the inconsistencies between the various results, both for the anaerobic and aerobic exercises, can be caused both by the training status, but also by the great inter-individual variability of the response to the oxidative stress induced by physical activity [3, 8].

2.2 Antioxidants and exercise

An active debate still exists on the effect of antioxidant supplementation on exercise-induced oxidative stress. Typical treatment generally includes vitamins A, C, and E, at various dosages, administered alone or in combination, chronically or acutely [15, 16]. Of these, vitamins C and E have been used more frequently in clinical and experimental studies, mostly because of their safety profile and easy availability [15]. One study showed the administration of vitamin C (500 mg, a moderate dose), reduced exercise-induced lipid peroxidation, and muscle damage in an untrained male group compared to the placebo group; on the contrary, it had no effect on inflammatory markers [17].

Other less-used antioxidants include coenzyme Q10 and N-acetylcysteine [15].

Regarding the endpoints, it is possible to hypothesize that the antioxidant could be effective in particular conditions in terms of training; for example, a specific moment of training (for example, before or after the race) or a type of sport compared to another (e.g., anaerobic versus aerobic).

Therefore, to decide whether to administer an antioxidant supplement, the selection and detailed description of the appropriate training stimulus and/or monitoring of the athlete during the training phases is necessary [15].

However, it is important to underline that numerous studies report negative effects of antioxidants. It is hypothesized that one of the motivations for the controversy is the different population analyzed in the studies.

In most of the studies in which a benefit of antioxidant supplementation was demonstrated in attenuating muscle damage and oxidative stress following endurance exercise, data on samples of sedentary and nonresistant subjects were analyzed. The endogenous antioxidant defenses of trained subjects could be over regulated and therefore these subjects may not benefit greatly from the use of exogenous antioxidants [15]. Furthermore, another big difference is represented by the different types of exercise; in fact, we move from the exercise of short-term resistance to the exercise of long-term resistance. In fact, aerobic endurance exercise will surely induce, due to the massive use of oxygen, a different flow of radicals with respect to the exercise of anaerobic resistance [15].

Recently, a large body of literature has highlighted a potential relationship between oxidative stress and the bioactive compounds present in plant foods. In particular, the researchers' attention has been shifted to the effects of a peculiar class of bioactive nutraceutical compounds, that is, polyphenols.

The study of phenolic compounds present in food has attracted great interest since the 1990s due to the growing evidence of their beneficial effect on human health. One of the first studies that stimulated the interest of scientists is the epidemiological study of Zutphen. In this research, an inverse association was proposed between the intake of foods rich in polyphenols and the incidence of diseases, such as diabetes mellitus, cardiovascular diseases, and cancer [18] and in particular of those pathologies associated with an evident oxidative stress.

Therefore, efforts to develop dietary strategies against oxidative stress caused by physical activity are being made and recently, there has been a growing interest in investigating the potential of polyphenols to modulate physical performance and prevent oxidative stress.

3. Polyphenols

Human diets are rich in polyphenols; western populations consume an estimated 1–2 g/day polyphenols, mainly from fruits, vegetables, and beverages such as tea, coffee, wine, and fruit juices. Polyphenols exert a range of biological activities, and various epidemiological studies and clinical trials have linked their intake with a reduced risk of chronic diseases, such as coronary heart disease, stroke, type II diabetes, and some cancers. There has recently been growing interest, supported by a number of epidemiological and experimental studies, on the possible beneficial effects of polyphenols on brain health.

For this reason, these phytochemicals are currently considered important components of a healthy diet, and it is believed that the health benefits of a diet rich in fruit and vegetables are to be attributed to these molecules. For example, the protective effects of tea against cardiovascular disease or coffee against type II diabetes could be explained by the large concentration of catechins present in these drinks.

As a consequence, the scientific and commercial interest in these phytochemicals has increased considerably in recent years; in particular, there are numerous works in which topics concerning their bioavailability, bioactivity, metabolism, and health effects have been addressed [19–21].

3.1 Nomenclature, classifications, and occurrence in foods

Polyphenols are classified into flavonoids and nonflavonoids, according to the number of phenol rings and structural elements bound to these rings.

Flavonoids represent the largest group of polyphenols. The chemical structure is characterized by 15 carbon atoms, derived from the flavone and all share certain properties. They are mainly soluble in water; usually, they are present in the plant as glycosides and in the same plant, there can be a flavonoid aglycone in combination with different sugars. Their name derives from flavus (=yellow) and refers to the role they play as plant pigments. The coloring that they give to the tissues depends on the pH. A specific group of flavonoids, the anthocyanins, is responsible for the red, blue, and violet colors of flowers and fruit and is therefore very important as a mediator of pollination. It is therefore not surprising that the variety of shades of color associated with anthocyanins has been increasing through the evolutionary process.

There are six dietary groups of flavonoids:

- flavones (luteolin and apigenin), which are found in parsley, capsicum pepper, and celery;
- flavonols (kaempferol, quercetin, and myricetin), which are found in onions, leeks and broccoli, cherry tomato, apple, berries, beans, tea and red wine;
- flavanones (hesperetin, naringenin, and eriodictyol), mainly present in orange, lemon juice, grapefruit, and herbs (oregano);
- isoflavones (genistein and daidzein), which are mainly found in soybeans;

- flavanols (catechin, epicatechin, and gallocatechin), abundant in green tea, apple, red wine, cocoa, chocolate and may be present as monomers or as oligomers; in particular flavan-3,4-diol polymerization produces so-called “condensed tannins”: they are also the origin of the catechins and probably polymerize with them to give the proanthocyanidins;
- anthocyanidins or anthocyanins (delphinidin, pelargonidin, cyanidin, malvidin, and petunidin), whose sources include red wine, berries, blackcurrant, and cherry [22–25].

The nonflavonoid group can be separated into three different classes: phenolic acids, stilbenes, and lignans.

Phenolic acids can be found in many plant species, in dried fruit. The most common phenolic acid are:

- caffeic acid is generally the most abundant phenolic acid and is mainly found as a quinic ester; present in many fruits and vegetables; it is a major phenolic compound in coffee;
- chlorogenic acid is the ester of caffeic acid and quinic acid; it is present in blueberries, kiwis, prunes, and apples;
- ferulic acid present in cereals, which is esterified to hemicellulose in the cell wall.

The best studied stilbene is resveratrol, and it can be found in *cis* or *trans*, or glucosylate, or in lower concentrations as the parent molecule of a family of polymers such as the food sources of viniferine, pallidol, or ampelopsin A. The resveratrol is found in particular in the grape skin and in the wine, to a greater extent in the red one.

Lignans (secoisolariciresinol, matairesinol, medioresinol, pinoresinol, and lariciresinol) are found in high concentration in linseed and in minor concentration in algae, leguminous plants, cereals, vegetables, and fruits [22–24].

To the large group of polyphenols are also added some molecules that form a separate category, compared to the four high. These include tyrosol and curcumin, which have long been discussed for potential health benefits.

The main classes of polyphenols present in the common diet are the flavanols, in particular the catechins and tannins of tea; the flavanones, mostly hesperidin present in citrus fruits; flavonols, such as quercetin in tea, apples, and onions; hydroxycinnamic acids, phenolic acids, abundant in coffee and many fruits and vegetables; anthocyanins, polyphenols responsible for the color of fruit and vegetables.

Among others, fruits like apples, grapes, pears, and berries typically contain high amounts of polyphenols (200–300 mg/100 g). Other polyphenol compound is curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a member of the curcuminoid family. It is found in turmeric, a spice produced from the rhizome of *Curcuma long*. Moreover, olive oil is a source of at least 30 phenolic compounds; the three phenolic compounds found in the highest concentration in olive oil are oleuropein, hydroxytyrosol, and tyrosol [21, 23].

Since agriculture was developed in 10,000 bc, humans have been modifying plant secondary metabolite profiles. By selecting fruit, flower, and vegetable colors, farmers involuntarily elected higher anthocyanin content, whereas in selecting for scents, they modified volatile phenolics. Anyway, as regards the content of polyphenols present in foods, it seems impossible to classify basic foods in terms of

“how many polyphenols they provide annually”. However, the most important food products, consumed in large quantities, which are the main source of polyphenols, are fruits and vegetables, green and black tea, red wine, coffee, chocolate, and extra virgin olive oil.

Even herbs and spices, nuts and algae are possible sources of food polyphenols; this depends on the culinary traditions and habits.

In 2010, a website was created (www.phenol-explorer.eu) containing over 35,000 content values for 500 different polyphenols in over 400 foods [16, 26].

Numerous factors can affect the content of polyphenols in food, may be environmental factors, such as exposure to the sun, precipitation, different types of crops, different types of soil, yield in fruits for the tree, degree of ripeness, but also conservation and methods of culinary preparation [27, 28].

3.2 Bioavailability of polyphenols

Bioavailability is defined as the fraction of a nutrient that the body is able to absorb and use for its physiological functions. Bioavailability may vary in relation to numerous factors, depending in part on the nature of the food and partly on the characteristics of the organism that assumes it. It is evident that bioavailability is very important in the nutritional field, but often neglected. A compound could have strong antioxidant activities or other biological activities *in vitro*, but if it were not bioavailable, if only a small amount of this type reached the target tissues, the molecule would have little biological activity *in vivo*. It is therefore essential to estimate the bioavailability of the polyphenols in order to set up effective nutritional protocols.

There are numerous studies carried out *in vitro*, then performed using as experimental models cultured cells or tissue slices, which provided fundamental information to understand the beneficial effects of polyphenols.

Despite the encouraging results, it is advisable to be very cautious in interpreting and extracting the data obtained in these experiments. Indeed, aglycons are often tested instead of active metabolites. Many researchers, when trying to unravel the physiological mechanisms involved in the health effects of polyphenols, often analyze the properties of a compound of little biological relevance. The dose used is also important, which should, in reality, be approximated to real life conditions. *In vitro* concentrations commonly range from low $\mu\text{mol/L}$ to mmol/L , while plasma metabolite concentrations, after a normal dietary intake, rarely exceed nmol/L . Using excessive doses in experiments performed *in vitro* could “force” positive outcomes; therefore, the results obtained must be extrapolated with great care in *in vivo* situations [23, 29].

3.3 Biological effects

Historically, polyphenols were mostly of interest to botanists, as they play many roles in plants. In the 1990s, polyphenols were classified as general antioxidants [30], and it was thought that it was easy to explain their activity. The reality of polyphenols is much more complex: biological effects involve detailed biochemical interactions with pathways at the molecular level. Much progress has been made in the last few decades.

Although the chemical structure gives polyphenols primarily an antioxidant activity, this property does not necessarily represent their biological effect, since any action on the organism depends on both bioavailability and molecular targets.

The overall effect of these phytochemicals on the reduction of disease risk is supported by epidemiology, where foods and beverages rich in polyphenols are

protective against the development of certain chronic diseases, in particular cardiovascular diseases, type 2 diabetes, and cancer [21, 31–35].

In recent years, revisions have been published supporting a new effect of polyphenols, that is, their role as supplements in athletic performance [11, 12].

However, despite these reviews, the overall effect of polyphenols on performance is inconclusive as most studies involve a small sample size.

Polyphenols are natural antioxidants present in the human diet in which they can reduce the damage due to the production of ROS. The chemical characteristics of the polyphenols allow them to act as direct scavengers of free radicals, such as the catechol group on the B ring, the presence of hydroxyl groups on the 3 and 5 position, and the 2,3-double bond in conjugation with a 4-oxofunction of a carbonyl group in the C-ring [9]. However, polyphenols can also behave as pro-oxidants at high doses or in the presence of metal ions, leading to DNA degradation. There is no evidence of systemic pro-oxidant effect of polyphenols in humans [21, 36]. Because of the low bioavailability and the kinetic constraints, the direct antioxidant activity of the polyphenols appears to be ineffective *in vivo*. Therefore, it has been hypothesized that the beneficial effects are not due to the direct scavenger properties on ROS, but to an indirect antioxidant action. In fact, polyphenols can modulate gene expression by inducing the endogenous antioxidant enzyme defense system.

4. Polyphenols action on athletic performance

As of today, a lot of studies are on polyphenols and physical exercise and in particular the polyphenolic compounds that have been demonstrated both to exert a significant effect in exercise-induced muscle damage and to play a biological/physiological role in improving physical performance. The effects of different polyphenols have been investigated in a wide range of exercise conditions, using a variety of supplementation strategies, timing, and dosage. Until a few years ago, despite the active search for “natural,” polyphenol-rich extracts that might enhance physical performance and decrease oxidative damage because they are antioxidants, the information we had was very limited and in some cases, they suggested the converse [37]. But in recent years, studies have increased considerably, and more information is now available on the effect of polyphenols on sports performance [14, 38–42].

4.1 Quercetin

Among nutraceutical compounds, flavonoids are the mainly studied ones for their positive effects on human health, and some of them have been proposed to be beneficial in exercise and exercise performance. Among flavonols, quercetin accounts for about 13.82 mg/day, resulting in being one of the most abundant flavonols in Western diet. Quercetin (3,4,5,7-pentahydroxyflavone) is a natural bioactive flavonoid, mainly present as quercetin glycosides (rutin, spiraeoside, troxerutin, quercitrin, isoquercetin, and hyperoside). It is distributed in a wide variety of natural foods, such as nuts, grapes, broccoli, and black tea; it is found in apples, berries, onions, grapes, and tomatoes as well as in some medicinal plants as *Hypericum perforatum* and *Ginkgo biloba* [42].

Antioxidant properties of quercetin are attributed to its chemical structure, particularly the presence and location of the hydroxyl (–OH) substitutions. Quercetin supplementation studies in athletes have focused on the potential effects of exercise-induced inflammation, oxidative stress, immune dysfunction, and exercise performance [42]. The first human exercise study investigating quercetin supplementation was published in 2006, with many more published in the past

few years and continuing to be published. When athletes are studied, most of the researches have failed to find an ergogenic effect, in contrast to that of a study of elite cyclists, who exhibited an improvement of their aerobic performance, and another study indicated that administration of quercetin (1200 mg) for 6 weeks resulted in performance improvement in cyclists [43].

The effects of quercetin supplementation in cycling athletes have been investigated [44]. Forty athletes were recruited and randomized to quercetin or placebo. Subjects consumed 1000 mg quercetin or placebo each day for 6 weeks before and during 3 days of cycling at 57% work maximum for 3 h. Despite previous data demonstrating potent antioxidant actions of quercetin in *in vitro* and animal models, long-term quercetin supplementation was not able to exert any preventive effect on exercise-induced oxidative stress and inflammation biomarkers. In another study, the influence of 1000 mg quercetin with or without 120 mg of epigallocatechin 3-gallate, 400 mg of isoquercetin and 400 mg of eicosapentaenoic acid and docosahexaenoic acid was evaluated on sports performance, biogenesis of muscle mitochondria and changes of markers of immunity and inflammation before and after a 3-day period of heavy effort. Two-week supplementation with polyphenols was effective in augmenting inflammation after 3 days of heavy exertion in trained cyclists. The feeding of untrained healthy men and women was supplemented with 1000 mg quercetin for 7 days, and the effect on VO_2 max and fatigue time was evaluated using a bicycle ergometer. Both fatigue (13.2%) and VO_2 max (3.9%) increases were found [42].

Other studies have analyzed the effect of quercetin on exercise performance, some reporting positive effects, while others do not, but to our knowledge, an increase in mitochondrial biogenesis has not been reported in human even though it has been shown a modest and insignificant increase in relative mitochondrial DNA copy number following quercetin supplementation [45].

A meta-analysis results have demonstrated that polyphenol supplementation for at least 7 days increases performance by 1.90%. Sub-analysis of seven studies using quercetin identified a performance increase of 2.82% [41]. There were no adverse effects reported in the studies in relation to the intervention. Polyphenol supplementation for at least 7 days has a clear moderate benefit on performance in healthy individuals. The performance benefits caused by quercetin supplementation are higher than those of other polyphenols. Further research is needed to confirm the optimal dose, even if a major intake could improve performance response.

Overall, the pooled results show that quercetin is viable supplement to improve performance in healthy individuals.

4.2 Catechins: green tea extract

Although quercetin is the most studied flavonoid in relation to exercise, other molecules are under investigation for their ability to prevent exercise-induced muscle damage and to affect physical performance. As of today, many studies on polyphenols and physical exercise concerned in supplementation with antioxidants like the green tea extract (GTE) from *Camellia sinensis*. GTE extract is rich in polyphenols, with flavonoid structure, including epigallocatechin gallate, epicatechin, epigallocatechin, and epicatechin gallate, which result in a powerful antioxidant activity [22–25]. Green tea supplementation has been advocated as a strategy to improve exercise recovery due to the activity of its catechins with high antioxidant and anti-inflammatory potential [46–48].

Although most studies on green tea have been performed in animals, a considerable amount of data is now available in humans. A green tea extract rich in catechins and caffeine increases the daily energy expenditure in humans. More recently, an

acute dose of green tea extract has been evaluated on healthy untrained men in a 30 min cycling test at 60% VO_2 max [46–48].

Other studies showed that GTE supplementation might reduce oxidative stress and promote improvement in the maximal oxygen uptake during cycling to exhaustion. Furthermore, GTE can reduce muscle soreness resultant of eccentric exercise and decrease markers of muscle damage after eccentric exercise, intense aerobic exercise, and strength exercises. Similar effects were not found when a single-dose of GTE was intake before intense muscle-endurance. The effects described for GTE supplementation suggest that GTE supplementation could be a valuable strategy for preserving performance during repeated periods of exercise that cause cumulative fatigue [47, 48].

Jowko et al. have tested the activities of green tea catechins in healthy individuals and soccer players and have been found to be very modest protection from oxidative damage in the first and no effect in the second [47].

Furthermore, it should be understood whether the supply of catechins increases or decreases the performance, in addition to the alleged cellular antioxidant activities. It has been tested a combination of epigallocatechin-gallate and N-acetylcysteine in healthy volunteers who performed eccentric exercise bouts [49]. In another study, the 4-week green tea extract supplement in previously untrained men increased the total plasma antioxidant potential and prevented oxidative damage. In another study, the protective effect of green tea drinks on oxidative stress and muscle damage parameters was also observed in weight-trained men [47, 48].

GTE supplementation protected against oxidative stress is induced by acute muscular endurance test, as well as against muscular damage induced by the training alone. Similar observations were reported in a study [50] about a group of resistance-trained men. In both cited studies, significant decrease in post-exercise plasma creatine kinase activity was noted as a result of supplementation.

However, GTE supplementation provided no protection from exercise-induced muscle damage. On the other hand, a number of previous studies revealed intensified muscle damage and hindered recovery as a result of antioxidant supplementation [47, 48].

Supplementation with GTE prevents oxidative stress induced by high-intensity repeated sprint test in male sprinters. On the other hand, neither protection from exercise-induced muscle damage, nor an improvement in sprint performance was noted after GTE intake. The use of GTE as a supplement is probably not useful in the case of sprinters, at least during the preparatory phase of their annual training cycle. Instead, the effects of taking GTE during the competition phase of the annual training cycle, being associated with a considerably greater exercise load, should be the subject of other research. Supplementation with green tea extract prevents oxidative stress induced by two repeated cycle sprint tests in sprinters. Furthermore, GTE supplementation does not seem to hinder training adaptation in antioxidant enzyme system. On the other hand, neither prevention of exercise-induced muscle damage, nor an improvement in sprint performance is noted after GTE administration [46].

Taken together, data from available studies seem to suggest that catechins can improve physical performance particularly in term of endurance capacity and VO_2 max in untrained subjects, and the same results could be reached in physically active people and well-trained athletes.

In conclusion, it is possible to state that supplementation of green tea extracts before a cumulative fatigue event minimizes muscle damage and oxidative stress in trained athletes. It also has positive effects on neuromuscular parameters related to muscle activation and muscle fatigue. Therefore, the use of GTE as a supplement can be considered a valid strategy in the context of competitive sport of resistance, which aims at the performance of athletes and the recovery of the exercises [48].

4.3 Resveratrol

At the beginning of the 90s, the idea was born that resveratrol, a compound present in red wine, could contribute in part to the “French paradox,” the presumed phenomenon for which in France, despite the relatively high consumption of foods rich in acids saturated fat, the incidence of mortality from cardiovascular disease was relatively low, lower than other dietetically comparable countries [51]. Resveratrol (3,4',5-trihydroxystilbene, RES) is a small polyphenol compound freely available in food supplements, and it is found in various berries, nuts, in the seeds and skins of grapes, red wine, mulberries, peanuts and rhubarb and other plants sources, including traditional Asian medicines [22, 23].

RES is an important activator of the sirtuin proteins and genes (SIRT, silent information regulators), causing an increase in the use of energy, and therefore, reinforcing the mitochondrial function. Sirtuins are silent, but significant regulators of metabolism, cancer, aging, and longevity; they are enzymes associated with the signal transduction pathways connected to stress [52].

Only few studies have investigated resveratrol ability in humans to modulate exercise performance, and some evidence suggests that it could play a role improving endurance capacity. There is a growing interest in the association between RES and exercise, because it has been hypothesized that the administration of RES can produce favorable effects on the rejuvenation of the liver cells, preserves the liver glycogen stores decreased during physical activity, and exercises a regulatory effect on glucose metabolism. To date, most of the studies that have investigated the effect of resveratrol administration on patient outcomes have been limited by their sample sizes.

In a study involving 14 athletes, RES supplementation was shown to inhibit the lipid peroxidation caused by exercise. In a study, it has been demonstrated that a combination of resveratrol and exercise training increased time to exhaustion compared to exercise training. The authors suggested that resveratrol optimizes fatty acid metabolism, which may contribute to the increased contractile force response of skeletal muscles [53]. Despite the inconsistency among reports regarding the topic, it has been suggested that RES delays fatigue by hindering lipid peroxidation, and recently there has been an interest in the capability of resveratrol to modulate physical performance and prevent oxidative stress. Currently, most clinical trials have been conducted with small samples, a wide range of dose levels and groups studied. As a result, it is difficult to establish a specific safety/efficacy range for the RES assay. Many conflicting discrepancies and information must be resolved before recommending the use of resveratrol as a supplement in sports performance [54].

4.4 Polyphenols mixtures

In recent years, the research has focused on studying not only the action of individual polyphenols but also the biological effects of polyphenols mixtures.

In muscular myotubes incubated with polyphenolic extracts of blueberry fruits (*Vaccinium corymbosum* cv. Reka), a dose-dependent protective effect on oxidative stress was observed [55].

The dark chocolate polyphenols were held responsible for some positive effects of dark chocolate consumption during the year. The effects of regular dark chocolate consumption (80 g/day for 2 weeks), rich in cocoa polyphenols, were analyzed on a sample of 20 active men. Plasma metabolites, hormones, and oxidative stress markers were evaluated after prolonged exercise. It has been observed that dark chocolate intake is associated with reduction of oxidative stress markers and increased mobilization of free fatty acids after exercise, but has no observed effect on exercise performance [56].

It has been found that *Ecklonia cava* (a species of brown alga present in the ocean of Japan and Korea) polyphenols acute preexercise supplementation induces a slight but significant increase in time to exhaustion in healthy human subjects [57].

Anthocyanins represent a class of polyphenols whose use is spreading among sportsmen. They are easy to find in berries and other colorful fruits and vegetables.

They can act as antioxidants and anti-inflammatory and therefore can improve recovery from exercise. *In vitro* observations showed anthocyanin-induced activation and endothelial nitric hormone metabolite metabolism and human vascular cell migration. The mechanisms by which anthocyanin intake can improve exercise performance may include effects on metabolic pathways, blood flow, and peripheral muscle fatigue, or a combination of all three. However, in general, the effects of these polyphenols on physical performance are less clear. For example, the use of black currant showed effects on the performance of the exercise; less noticeable effects have instead been analyzed after a cherry intake. Therefore, probably, the benefits could be due to specific food-dependent anthocyanins [12, 14].

Yerba Mate (YM) is a South American plant, rich in polyphenols, saponins, and xanthines, of growing scientific interest because of its metabolic effects. YM has been shown to increase fat utilization during exercise in untrained humans. Its metabolic and physical performance effects were characterized in 11 well-trained male cyclists. YM increased fat utilization during submaximal exercise and improved time trial performance [58].

Montmorency cherry concentrate is used as a supplement by the athletes of the Australian Institute, because it is rich in anthocyanidins. These cherries possess high anti-inflammatory and antioxidant capabilities, can improve sleep and reduce muscle damage and post-exercise pain [59].

Exercise-based studies evaluated the effects of cherry juice supplementation on recovery from maximum strength or resistance (duration > 60 min), demonstrating the attenuation of markers related to both inflammation and oxidative stress [60].

Any response linked to accelerated recovery would appear beneficial when considering the large training load experienced by high performance athletes. In reverse, cherry juice (CJ) supplementation had no significant effect on the recovery of Water Polo specific athletic performance. Probably, CJ supplementation may not be necessary for water-based nonweight bearing intermittent sports such as Water Polo [61].

Fourteen male students drank 12 fl oz. of a cherry juice blend or a placebo twice a day for eight consecutive days. A bout of eccentric elbow flexion contractions was performed on the fourth day of supplementation. Isometric elbow flexion strength, pain, muscle tenderness, and relaxed elbow angle were recorded before and for 4 days after the eccentric exercise. Strength loss and pain were significantly less in the cherry juice trial versus placebo. These results show efficacy of the cherry juice in decreasing some of the symptoms of exercise-induced muscle damage [62]. Another study has demonstrated that Montmorency cherry juice consumption improved the recovery of isometric muscle strength after intensive exercise [63].

Regardless, future research should examine the use of CJ in other team sports before CJ can be recommended or excluded as an integrator to improve recovery after sport performance.

Blackcurrant (*Ribes nigrum*) fruits are a real mine of polyphenols, in fact they are rich in anthocyanins delphinidin-3-rutinoside, delphinidine-3-glucoside, cyanidin-3-rutinoside, and cyanidin-3-glucoside. The health benefits are thought to be mediated by the effect of anthocyanins on inflammatory responses, antioxidant activity, and endothelial function [64]. Moreover, blackcurrant intake increases forearm blood flow at rest, potentially mediated by anthocyanin-induced vasodilation and vaso-relaxation which may affect substrate delivery and exercise performance. It is

important to emphasize that the blackcurrants properties are common to all berries (raspberry, blueberry, blackberry, currants, and gooseberries).

Recent studies have revealed a potential ergogenic effect of New Zealand blackcurrant (NZBC) extract intake on physiological and metabolic exercise responses and performance outcomes. In one study, the effect of New Zealand's blackcurrant extract on performance during anaerobic sprint test running in youthful and recreational male soccer players was evaluated. A clear benefit of NZBC's short-term intake on fat oxidation and physical performance has been demonstrated, and the extract seems to benefit the repeated sprint performance only in trained players [65]. Moreover, 7 day NZBC intake augments fat oxidation during 120 min moderate-intensity exercise in endurance-trained females [66].

Another polyphenols-rich fruit is pomegranate; in fact, pomegranate juice (POMj) is rich in flavonols, flavonoids, gallic acid, ellagic acid, quercetin, and ellagitannins, with numerous health benefits during stressful situations [67].

Its antioxidant potential has proven to be superior even to green tea and red wine. According to recent studies, in fact, the pomegranate reduces oxidative stress of macrophages, free radicals, lipid peroxidation, and oxidation of low-density lipoproteins; the inflammatory processes seem to be blocked by the action of ellagitannins. Pomegranate juice is an excellent post-workout because the antioxidants present in the juice of the arils help the muscles to restore their functionality facilitating the supercompensation of exercise; it has a significant impact on acute post-exercise lipid peroxidation and on enzymatic and nonenzymatic antioxidant responses.

Pomegranate extract has been suggested as an ergogenic aid due to its rich concentration of polyphenols, which are proposed to enhance nitric oxide bioavailability, thereby improving the efficiency of oxygen usage, and consequently, endurance exercise performance. Supplementation with pomegranate juice has the potential to attenuate oxidative stress by enhancing antioxidant responses assessed acutely and up to 48 h following an intensive weightlifting training session [68, 69].

The polyphenol *curcumin*, derived from the rhizome *Curcuma longa* L., is a natural antioxidant that exhibits various pharmacological activities and therapeutic properties and has been used to treat a variety of inflammatory conditions and chronic diseases. It has been demonstrated that curcumin can reduce the accumulation of advanced glycation end-products *in vitro* and in animal models, suggesting that this anti-glycation mechanism may relate to the antioxidant effect of the compound. It has been suggested a positive effect of curcumin and *Boswellia serrata* gum resin supplementation for 3 months on glycoxidation and lipid peroxidation in athletes chronically exercising intensively and further studies will test whether treatment with curcumin can result in a reduction of the accumulation of advanced glycation end-products in muscle tissue, possibly improving muscle performance in the long term [70]. It has been demonstrated that consumption of curcumin reduced biological inflammation, but not quadriceps muscle soreness, during recovery after exercise-induced muscle damage. The observed improvements in biological inflammation may translate to faster recovery and improved functional capacity during subsequent exercise sessions [71].

Honey, natural food produced by the nectar of flowers from bees, is widely used for its precious nutritional and therapeutic values that provide phytotherapeutic properties, with powerful antioxidant, anti-inflammatory, and antimicrobial effects. So far, around 300 types of honey have been recognized with different taste, color, and odor according to the different types of nectar harvested by bees. Honey is an ancient *nutraceutical*, which owes its properties to the richness of polyphenols, which vary according to the floral variety from which it derives, but in general it is made up of flavonoids, between 50 and 500 mg/kg, including

galangina, quercetin, kaempferol, and luteolin, which represent the bioactive molecules with a strong antioxidant action.

Honey is an energizing substance useful for sportsmen, and it provides up to 17 g of carbohydrates for every spoon consumed and provides the much needed energy, serving as an economic substitute for the enhancers of sporting activities available on the market. A beneficial effect of honey has been shown in athletes, where if a moderate and regular exercise is able to counteract oxidative stress [20]. In one study, 32 healthy volunteers underwent a short but intense exercise on the ergometer. A significant decrease in serum malondialdehyde levels was observed in subjects who had consumed honey before making a physical effort, with a greater difference for those volunteers who had used it for 3 weeks.

In another study, the effects of honey in 39 road cyclists were examined. In the group that received honey supplementation (70 g), the increase in oxidative stress markers was much lower than placebo, and the antioxidant levels were significantly higher. Ahmad and others examined the effect of different doses of Tualang honey in 20 athletes involved in different competitive sports. The results showed that there was no significant difference between the two different doses and that the maximum antioxidant capacity was observed in both cases 2 h after the honey intake [20].

5. Polyphenols supplementation in exercise: limits and considerations

The use of polyphenols has been designed to improve performance by increasing mitochondrial biogenesis in two ways: polyphenols stimulate stress-related cell signaling pathways that increase the expression of genes encoding cytoprotective proteins such as nuclear respiratory factor; the selected polyphenols (i.e., resveratrol, curcumin, and quercetin) have been reported to modulate muscle function and mitochondrial biogenesis by activating the sirtuins and increasing the activity of the c-receptor co-activator activated by the peroxisome proliferator. Furthermore, some polyphenols improve flow-mediated dilation and endothelial function in humans by increasing the synthesis of endothelial nitric oxide. Polyphenols could help overall athletic performance in sports where the rate of blood flow and maximum cardiac output are important determinants of cardiovascular performance, acting on endothelial function.

Polyphenol supplementation is currently controversial, and at the moment, the use of different exercise protocols, different outcomes, in various physically trained subjects, and the use of a variety of laboratory parameters to demonstrate these effects make it still difficult to assess the effects of polyphenols on physical activity. Therefore, in any case, a detailed description of the type of exercise (e.g., aerobic or anaerobic), the oxidative stress biomarkers used, the characteristics of the subject and the training endpoints examined to allow data interpretation is always necessary.

The evidence is not sufficient to make recommendations for or against the use of polyphenol supplements for recreational, competitive, or elite athletes. Polyphenols have multiple biological effects, and future exercise studies must be studied in an appropriate and specific way to determine the physiological interactions between the exercise and the selected supplement, rather than considering only performance.

Those with higher levels of oxidative stress can clearly benefit more from the antioxidant treatment. An initial screening of the state of oxidative stress is therefore essential. Clearly, individual susceptibility related to the presence of specific genetic variants in key enzymes for ROS detoxification may be another important parameter.

It would be useful to consider the integrated effect of exogenous diet and antioxidant supplementation.

6. Conclusion

The relationship between oxidative stress and sport is really very complex; in fact, the release of free radicals is necessary to stimulate the up-regulation of endogenous antioxidant defenses. In recent years, the consumption of supplements rich in antioxidant compounds by athletes has greatly increased, but a natural intake through the diet is more recommended.

For future research conducted on the performance effects of dietary polyphenols, it should provide adequate detail on the method of blinding and participant follow-up to ascertain whether the study was in fact blinded and report performance data in raw values. These designs would enable researchers to optimize both type and dose of polyphenol supplementation to achieve performance benefit. In addition to the general notes on research reporting, very few studies outlined comprehensive dietary control measures.

Researchers should attempt to quantify the participants' dietary intake of polyphenols, as those with low intakes are likely to respond more favorably to dietary intervention.

Polyphenol supplementation for at least 7 days has a clear moderate benefit on performance in healthy individuals. More research is needed on optimal dose; however, greater intakes could improve the performance response.

The present review summarized the results of studies on the effects of polyphenols intake on exercise-induced oxidative stress obtained in human trials.

The conflicting findings of previous research have brought into question the usefulness of antioxidant supplementation during resistance training. As polyphenolic antioxidants have shown promise as recovery strategies from fatiguing and damaging bouts of exercise, supplementation with polyphenols may be an appealing option to recover from an intense resistance exercise bout. However, it is important to determine whether polyphenol supplementation during a resistance training program will augment or diminish adaptations in muscular strength. Clearly, there is much more to be learned in the exciting field of exercise, oxidative stress, and polyphenols.

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Conflict of interest

The author declares that there is no conflict of interest.

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