Effects of pomegranate supplementation on exercise performance and post-exercise recovery in healthy adults: A systematic review

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Abstract

The functional significance of pomegranate (POM) supplementation on physiological responses during and following exercise is currently unclear. This systematic review aimed (i) to evaluate the existing literature assessing the effects of POM supplementation on exercise performance and recovery; exercise-induced muscle damage, oxidative stress, inflammation; and cardiovascular function in healthy adults and (ii) to outline the experimental conditions in which POM supplementation is more or less likely to benefit exercise performance and/or recovery. Multiple electronic databases were used to search for studies examining the effects of POM intake on physiological responses during and/or following exercise in healthy adult. Articles were included in the review if they investigated the effects of an acute or chronic POM supplementation on exercise performance, recovery and/or physiological responses during or following exercise. The existing evidence suggests that POM supplementation has the potential to confer antioxidant and anti-inflammatory effects during and following exercise, to improve cardiovascular responses during exercise, and to enhance endurance and strength performance and post-exercise recovery. However, the beneficial effects of POM supplementation appeared to be less likely when (i) unilateral eccentric exercise was employed, (ii) the POM administered was not rich in polyphenols (< 1.69g/L), and (iii) insufficient time was provided between POM-ingestion and the assessment of physiological responses/performance (≤1h). The review indicates that POM has the potential to enhance exercise performance and to expedite recovery from intensive exercise. The findings and recommendations from this review may help to optimize POM-supplementation practice in athletes and coaches to potentially improve exercise-performance and post-exercise recovery.

Keywords: sports nutrition; polyphenol; oxidative stress; muscle damage; inflammation.

1 Introduction

Pomegranate (POM) or Punica granatum is an ancient fruit originating from the Middle East [1]. The POM fruit is berry-like with a leathery rind enclosing many seeds surrounded by the juicy arils, which comprise the edible portion of the fruit [2]. This edible part represents ~ 52% of total fruit weight, comprising 78% juice and 22% seeds [3]. Dietary supplementation with POM fruit has traditionally been consumed as POM juice (POMj) obtained from the first-press (partial pressing) squeezing of whole pomegranate fruits [4]. More recently, POM extract (POMe) has been developed in liquid and dry powder forms to provide alternative convenient sources for obtaining the bioactive polyphenols found in POMj. The liquid POMe is produced by extraction of the remaining fruit residue obtained, after an additional pressing, and the powdered POMe is obtained from further resin purification and drying (solid-phase extraction) to produce a powder with a high concentration of polyphenols [4].

Dietary supplementation with POMj or POMe, which are both rich in polyphenols, has been reported to promote several beneficial health effects [5,6]. In particular, POM supplementation appears to be effective at enhancing physiological responses in individuals exhibiting physiological stress such as cardiovascular disease [7], oxidative stress [8], cellular inflammation [9] or joint or muscle damage [10,11]. Indeed, POM consumption has been reported to lower cardiovascular disease morbidity by enhancing myocardial blood flow (+17%) [12] and antioxidant status (+130%) [7], and lowering low-density lipoprotein cholesterol oxidation (-90%), systolic blood pressure (-12%), and carotid artery thickness (-30%) [7].Similarly, POM has been shown to attenuate oxidative stress by lowering free radical production and lipid peroxidation (-65%) [13], and to inhibit some cellular inflammation transcripts [14,15] such as nuclear factor -κB (NF-κB), tumor necrosis factor α (TNFα) and cyclooxygenase-2 (COX -2). Since, these positive physiological effects afforded by POM supplementation have the potential to prevent or treat various disease risk factors, POM has been described as a "super fruit" [14]. In this context, and compared to other purported nutraceuticals (e.g., green tea, red wine, orange, blueberry and cranberry juices), POM supplements have been reported to confer the most potent antioxidant and anti-inflammatory effects [13, 16]. Indeed, compared to the aforementioned foods, POMj is more effective in attenuating low density lipoprotein (LDL) oxidation and inhibiting cellular oxidative stress in macrophages. Moreover, POMj exhibits a high capacity to neutralize free radicals with a reported antioxidant activity three times higher than of red wine and green tea (Trolox equivalent antioxidant capacity=18-20 vs. 6-8) [16]. POM also possesses a higher antioxidant activity compared to other food stuffs such as turmeric, ragi, amla, amaranth, rajmah, sesame, wheat and flaxseed [13]. Although the underlying mechanisms for the beneficial physiological effects of POM supplementation are not yet clear [17-19], its efficacy has been attributed to the high bioavailability of its constituent polyphenols compared to other polyphenol-rich foods [5,16].

Physical exercise is a potent and multifaceted physiological stressor, as evidenced by an immediate increase in markers of muscle damage [20-23], inflammation and oxidative stress [23,24] and a protracted period of muscle weakness and soreness during the post-exercise recovery period [17, 25]. Since POM supplementation appears particularly effective at improving numerous physiological responses in individuals manifesting symptoms of physiological stress [7, 12-14], POM supplementation might have potential as an ergogenic and recovery aid. Notwithstanding this potential for enhanced exercise performance and post-exercise recovery following POM supplementation, studies assessing the effects of POM supplementation on exercise performance and recovery are limited and yield equivocal findings [17-19, 25-27].

The aims of the present systematic review were (i) to examine the effect of POM intake on exercise performance and recovery, as well as its acute and delayed effects on muscle damage, oxidative stress, inflammation and cardiovascular function following exercise in healthy individuals, and (ii) to outline how aspects of the study design (e.g., fitness status of participants, biomarkers assessed, supplement dose and exercise protocol) can influence the potential ergogenic and recuperative effects of POM supplementation. The recommendations from this review will have the potential to inform POM supplementation guidelines to optimize exercise performance and recovery practices in athletes and sports nutritionist.

2 Methods

This systematic review was conducted and reported in accordance with the guidelines of the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement, which is an evidence-based protocol describing a set of items for reporting in systematic reviews and meta-analyses [28].

2.1 Data Sources and Search Strategy

To inform our review, a comprehensive systematic search of studies was performed electronically in the following databases: PubMed / Medline, Web of Science and science direct from inception to January 2018. The search was limited to English language. The following search terms and Medical Subject Headings (MeSH) were used to source articles from pertinent peer-reviewed journals: Pomegranates (MeSH) OR Pomegranates (All Fields) OR Pomegranate (All Fields) OR Punicagranatum (All Fields) OR Punicagranatums (All Fields) OR granatum, Punica (All Fields) AND exercise (MeSH) OR exercise (All Fields) OR exercises (All Fields). The search was supplemented by manually cross-matching reference lists, key author searches, and citation searching of all retrieved papers to potentially identify additional studies. The search strategies were combined, and duplicates were removed by Endnote and manually by two of the authors. Once all relevant articles had been located, the researcher individually considered each article for its

appropriateness for inclusion based on the pre-determined inclusion criteria described below. Where there was uncertainty with regard to inclusion, discussion with a third researcher determined the final inclusion or exclusion of the article.

2.2 Inclusion and Exclusion Criteria

To be included in the systematic review, individual studies needed to fulfill the following inclusion criteria: (i) primary research published in peer-reviewed journals in English, (ii) research conducted with healthy human participants (sedentary, active or trained subjects), (iii) original studies that had investigated an acute or long-term POM supplementation intervention (juice or extract) on performance and/or physiological responses, (iv) no severe methodological deficiencies (e.g., absence of placebo control, participant were not blinded, inappropriate statistical analysis procedures) and (v) published before February 2018. Exclusion criteria were: (i) studies written in languages other than English, (ii) data from congress or workshop publications, (iii) animal studies, (iv) studies in which no supplementation was given, (v) studies which administered multiple supplements in addition to POM as this thwarted clear separation of the effects of POM from the other supplements, (vi) studies in which no exercise was performed, and (vii) studies in which exercise was performed in extreme environments (e.g., altitude, heat etc). No limits were set for the year of publication. Case studies, encyclopedia, book chapters and reviews were excluded, although the bibliographies of the latter were consulted to refine article searches.

2.3 Study Selection

Following the removal of duplicate studies from the different search engines, inclusion or exclusion of the remaining articles was performed by applying the above criteria on the title and abstract to determine eligibility in a preliminary independent screening. Selected papers were then read in full to finalize eligibility or exclusion. A summary of this process is outlined in Figure 1. The university's library, hand searches, electronical databases, and contact with the authors were used to obtain full copies of the published manuscripts.

2.4 Data Extraction

Data were extracted using a standardized form. The primary outcomes extracted in this review were the effects of POM supplementation on physical performance, fatigue, and perception of pain and soreness [e.g., rating of perceived exertion (RPE), delayed onset muscle soreness (DOMS), pain scale] during and/or following exercise. These outcomes are presented in Table 1. All data concerning the effect of POM supplementation on muscle damage [e.g., the concentrations of creatine kinase (CK), lactate dehydrogenase (LDH), myoglobin (MB), aspartate aminotransferase

(ASAT)], oxidative stress [e.g., thiobarbituric acid-reactive substances (TBARS), malondialdehyde (MDA), protein carbonyl (PC), total antioxidant capacity (TAC), glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), uric acid (UA), arylesterase (ARE)], inflammatory [e.g., c-reactive protein (CRP), high sensitive (hs-CRP), interleukin (IL-6), matrix metalloproteinases (MMP), sE-selectin, white blood cell (WBC)] and cardiovascular [e.g., heart rate (HR), blood pressure (BP), blood flow, vessel diameter, oxygen saturation (S_PO_2)] responses following exercise were extracted from the research papers and are shown in Table 2. For all extracted performance and physiological data (Tables 1 and 2), the effects of POM supplementation were separated into data collected (i) during and immediately (up to 2 hours) after exercise, which we classified as acute responses, and (ii) after a period of at least 24h/48h following exercise, which we classified as delayed responses [17-19, 22-25].

2.5 Quality assessment

To assess the methodological quality of the selected studies, the Physiotherapy Evidence Database (PEDro) scale was used [29]. The PEDro scale is based on the Delphi list developed by Verhagen and colleagues at the Department of Epidemiology, University of Maastricht [30]. The PEDro scale is a reliable and objective tool that helps identify which of the randomized clinical trials from the same areas of physiotherapy practice are likely to be externally (criteria 1) and internally (criteria 2-9) valid and could have sufficient statistical information to make their results interpretable (criteria 10-11) [29]. Each paper was independently assessed twice by two independent reviewers using the 11-item checklist to yield a maximum score of 10 (the sum of awarded points for criteria 2-11). Points are only awarded when a criterion is clearly satisfied. In case of disagreements concerning trial scoring, a discussion with a third reviewer was conducted. The level of agreement between reviewers was calculated via the Kappa values with k=0.91 indicating an excellent agreement [31].

3 Results

Eleven studies [17-19, 25-27, 32-36] met the inclusion criteria and were included in the current systematic review. The studies examined either the effects of POM intake on exercise performance and/or exercise-induced fatigue, soreness, muscle damage, oxidative stress, inflammation and cardiovascular function. All studies used a statistical significance threshold of $p<0.05$.

3.1 Study Selection and Characteristics

3.1.1 Study selection

188 The predefined search strategies yielded a preliminary pool of 786 possible papers. Removal of duplicates resulted in a
189 selection of 497 published papers. A first screening of titles and abstracts for eligibility against inclusion and exclusion
190 criteria let to a provisional list of 16 published studies. The full texts of 14 articles were retrieved, while two studies
191 were excluded because insufficient data were published. After a careful review of the 14 full texts, 3 articles were
192 excluded (2 studies investigated physical exercises performed in extreme conditions (i.e., altitude, heat) and one study
193 used POM combined with other supplements). Therefore, eleven studies met our inclusion criteria for determining the
194 effects of POM supplementation on exercise performance, recovery and a variety of physiological outcome
195 measurements.

196 ***3.1.2 Study characteristics***

197 The characteristics of each study, and the performance and the physiological changes following POM supplementation
198 compared to PLA supplementation, are respectively summarized in Tables 1 and 2. Four papers examined the effect of
199 POM supplementation on physical performance and physiological responses, such as muscle damage and inflammation,
200 following strength exercise [18, 25]; and cardiovascular responses following running [32], cycling [35] and strength [18, 35]
201 exercise. Two studies only examined the change in physical performance without physiological measurements [17, 33],
202 while the remaining five studies only assessed the effect of POM supplementation on the physiological responses to
203 exercise such as muscle damage [26], oxidative stress [19, 27, 34, 36], inflammation [26, 27] and cardiovascular function [34].
204 Different exercise models were employed in the studies included in the current systematic review. Specifically, four
205 studies included strength exercises such as unilateral eccentric [17, 25] and Olympic weightlifting [18,19] movements, three
206 studies employed treadmill running [27, 28, 34], two studies used a combination of strength and running [33] or cycling [35]
207 exercise, while the two remaining trials used ultra-endurance exercises [26, 36]. Further measures completed to assess the
208 physiological effects of POM supplementation included RPE [18], perceptions of DOMS [17, 18, 25] and pain and vitality
209 scales [28], which are presented with performance in Table 1. Concerning the acute (up to 2 hours) and delayed (at least
210 24/48h) responses to exercise, four studies assessed the acute and delayed performance and/or physiological responses
211 [17-19, 25], five studies only assessed the acute responses [27, 32, 34-36], while two studies [26, 33] only assessed the delayed
212 responses.
213
214 ***3.1.3 Subjects characteristics***

215 The studies involved in this systematic review included a total of 230 participants (190 males, 20 females, with 20 not
216 specified). The number of participants in each trial ranged from 9 [18, 19] to 45 [37], with a mean sample size of 20.9 ± 10.1
217 and a mean age ranging from 21 [17-19] to 35 [26] yrs. These 11 studies targeted healthy adult participants of varying
218 fitness status. Four studies recruited recreationally- [25, 33, 34] to highly- [32] active participants (total n=100participants),

four studies [17-19, 35] recruited resistance trained participants (n=54 participants), and three studies [26, 27, 36] recruited endurance trained athletes (total n=74).

3.1.4 Study design and supplement administration

As presented in Tables 1 and 2, the reviewed studies (nine out of eleven) implemented a double-blind, placebo (PLA)-controlled experimental design. The majority of these studies (nine out of eleven) employed a randomized design where (i) two studies employed three experimental arms [26, 33] with at least one being POM treatment, (ii) three studies used two experimental arms [27, 34, 36], and (iii) four studies used one experimental arm (i.e., crossover design) with a 1 week [32, 35] or a 2 week washout period [17, 25]. Concerning, the two remaining studies [18, 19], the experimental protocol was completed during one week of an intensive training program in a group of elite weightlifters (i.e., one experimental arm) which necessitated a small washout period (48h). Therefore, to avoid any possible protracted effect of POM supplementation on the physiological responses post training, the authors selected a non-randomized crossover design with the POM treatment administered first for all participants. The eleven trials included in this review employed one of two varieties of dietary POM supplementation with an intervention period that ranged from 30 minutes to 21 days. The majority (n=9) opted for POMj, with beverages ingested both prior to and following the training/exercises sessions. Indeed in 5 studies participants were supplemented for 4 days pre- and 4/5 days post exercise with 0.5L POMj once or twice daily [25, 33]; 7 days pre- and 8 days post-exercise with 0.25L twice daily [17]; 1h (0.5L) pre- and 2 days (3×0.25L/d) post exercise [18,19]. In the remaining four studies, POM was only ingested prior to exercises sessions with a treatment of: 0.5L/day during a period of 1 week [34]; 0.24L/day during a period of 2 weeks [27, 36]; or 0.2L/day during a period of 3 weeks [26]. The two remaining studies [32, 35] opted for an acute consumption of 1000mg POM extract 30 min prior to exercises sessions. With regard to the antioxidant capacity of the POM supplements administered in the selected studies, the total phenolic content ranged from 0.65g/0.5L [17, 25, 33] to 2.56g/0.5L [18, 19].

3.1.5 Methodological quality of studies

All reviewed studies scored a moderate to high score of 7 and above with a mean PEDro score of 8.9±0.9. Of the 11 studies included, 3 investigations [17, 25, 35] received a perfect score of 10 , 5 investigations [18, 19, 32-34] scored 9 out of 10 as they failed to randomly allocate subjects to a group or failed to achieve similar baseline values for the primary outcome measure, 2 investigations [26,27] scored 8 out of 10 as they failed to blind therapists and achieve similar baseline values for the primary outcome measure, and the remaining investigation [36] scored 7 out of 10 as the authors failed to achieve similar baseline values for the primary outcome measure and to blind the experimenters to the supplement order. Overall, the study quality was deemed to be good to excellent.

3.2 Effect of POM on acute and delayed physical performance

A total of six studies assessed the effect of POM supplementation on exercise performance [17,18, 25, 32, 33, 35]. Three of these studies evaluated the change in acute (immediately and up to 2hours) physical performance after treadmill [32], repeated sprint ability (RSA) [35] and strength [18, 35] exercise with the remaining three studies assessing the delayed (i.e., after a period of at least 24h/48h following exercise) effect of POM on strength recovery following unilateral [17, 25] and bilateral eccentric exercise [33].

3.2.1 Effect on acute physical performance

In highly active participants, ingestion of 1000mg of POMe (2×500mg capsules) 30min prior to exercise (Table 1) was reported to improve time to exhaustion (TTE) during treadmill running at 90% (388±199 vs. 346±163 s) and 100%, (171±66 vs. 159±62 s) but not 110% (108±45 vs. 104±40 s) of the peak velocity (PV) obtained in a graded treadmill test continued until exhaustion [32]. Moreover, the average and peak power output in sprint 5 during an RSA test on a friction-braked cycle ergometer (i.e., 6s maximal sprints × 10 repetitions with a load of 65g/kg of body mass applied and a 30s passive recovery separating intervals) was also enhanced following the same POMe ingestion procedures [35]. With regard to resistance exercise performance, POMe ingestion has been reported to have no effect on the number of repetitions to fatigue (RTF) during bench and leg press exercise [35]. Conversely, Ammar et al. [18] recently showed that consumption of 500 mL POMj 60 min prior to high-intensity weightlifting exercise enhanced the total (8.3%) and maximal (3.26%) load lifted in two Olympic movements (Snatch and Clean & Jerk) compared to the PLA condition (Table 1). The discrepancies between studies could be linked to inter-study differences in the supplementation procedures employed. Therefore, the existing findings suggest that ingestion of 500 mL of POMj 60 min prior to exercise is more likely to enhance resistance exercise performance than 1000 mg of POMe ingested 30 min prior to exercise.

The enhanced performance following POM ingestion might be linked to increased muscle blood flow. Indeed, Trexler et al. [32] observed enhanced performance in association with an increase in post exercise vessel diameter and brachial artery blood flow after POMe ingestion. However, given that blood flow was only investigated post-exercise at the brachial artery in this study, it still unclear whether POM increases arm and/or leg blood flow during exercise. The beneficial effect of POM on blood flow could be due to its high content of polyphenols (e.g., flavonoids) which can promote nitric oxide (NO) synthesis, (an important contributor to exercise-induced vasodilation [37]) by enhancing nitric oxide synthase (NOS) activity, and NO bioavailability, through limiting NO scavenging by reactive oxygen species (ROS) [38].

3.2.2 Effects of POM on muscle strength recovery

The performance of eccentric exercise has been shown to reduce maximal strength and increase the sensation of soreness in the exercising muscles, with muscle soreness peaking 24–48h post such exercise [39]. Although, soreness scores return towards baseline after this point [40], strength can remain depressed compared to baseline even up to several days after undertaking eccentric exercise [41]. It has been reported that full recovery of strength typically requires 7 to 14 days [42]. To date, studies assessing the effect of POM supplementation on post exercise muscle recovery (Table 1) have shown that, in both untrained [25] and trained [17] subjects, consumption of 500 mL POMj for 9 to 15 days prior to an intensified training session (2 to 3 sets of 20 unilateral maximal eccentric elbow flexion) can expedite the recovery of strength assessed during the 2-168h period post exercise. Indeed, compared to PLA, there was greater strength recovery following POMj supplementation at 48h (85% vs 78%) and 72h (89% vs 84%) post exercise [25]. Concerning the effect of POMj supplementation on lower limb recovery, Trombold et al. [17] showed that the recovery of knee extensor isometric strength was not affected by POMj after 6 sets of 10 unilateral eccentric knee extension exercise performed by resistance trained men. Collectively, these initial studies suggested that POM supplementation can accelerate strength recovery in arm muscles but not leg muscles. More recently, however, Machin et al. [33] showed that consuming POMj either once-daily (650 mg/d) or twice-daily (1300 mg /d) improved strength recovery in both leg and arm muscles after completing unaccustomed eccentric exercise in recreationally active men (Table 1). These conflicting results could be explained by the training status of the participants (untrained vs. resistance trained subjects) and/or the composition of the eccentric exercise protocols. Specifically, the eccentric exercise protocol employed by Machin et al. [33] was based on 20 min of downhill running, thereby engaging both sets of leg muscles and provoking a greater degree of physiological perturbation [43], whereas Trombold et al. [17] used a protocol comprising 6 sets of 10 eccentric unilateral knee extension exercises. The beneficial effect of POM on muscle strength recovery has recently been related to its antioxidant and anti-inflammatory properties [18, 19] and its ability to enhance vasodilation and blood flow [32, 37].

3.3 Effect of POM on muscle fatigue, pain and soreness

Four studies have examined the effects of POM on muscle fatigue, pain and soreness following physical exercise [17, 18, 25, 32]. Three of these studies analyzed the change in muscle fatigue and soreness acutely and up to 48h [18] or 96h [17, 25] post strength exercise, while only one study focused on the effect of POM on muscle pain immediately following treadmill runs session [32].

3.3.1 Effect on acute muscle fatigue, pain and soreness

In untrained subjects (Table 1), a daily drink of POMj prior to (4days) and following (4days) intense upper body eccentric exercise has been reported to lower the perception of muscle soreness in the elbow-flexors120min post-exercise [25]. Similarly, POMj consumed 1h before and over the 48h following a resistance training session (Table 1) has been reported to blunt the acute perception of muscle fatigue with lower ratings of perceived exertion (RPE) values reported (-4.37%) following POMj supplementation [18]. The immediate lowering of post-exercise muscle fatigue and soreness following POMj supplementation might be explained by blunted tissue oedema and/or a lower accumulation of metabolic by-products which relay information to the central nervous system via group III and IV muscle afferents [44]. This reduction in muscle soreness and fatigue following POMj supplementation might be expected to translate into less fatigue in a subsequent training session, which may have implications for enhancing physical performance during a training programme [22, 24]. With regard to the effect of POM supplementation on the perception of muscle soreness and fatigue following intermittent exercise, it has been reported that pain, as assessed using the visual analog pain scale, was not significantly affected by POMe treatment [32]. However, the following statement on the vitality scale, "At this moment I feel alive and vital", was found to be greater 30 min following POMe ingestion [32]. Taken together, these results indicate that POM supplementation appears to acutely attenuate the sensation of fatigue and soreness post exercise with potential implications for performance in subsequent training sessions.

3.3.2 Effect on delayed onset muscle soreness

Exhaustive or unaccustomed intense exercise can cause muscle damage, which results in pain, tenderness, swelling and stiffness. Given the delayed nature of these symptoms, they are collectively referred to as delayed onset muscle soreness (DOMS) [45]. Trombold et al. [17, 25] were the first to assess the effect of POMj supplementation on the DOMS provoked by a bout of intense eccentric exercise (Table 1). These studies showed that consumption of 250-500mL POMj twice daily could attenuate elbow flexor muscle soreness at 48 and 72 h post exercise in resistance trained males [17], but not in recreationally active males [25]. However, knee extensor muscle soreness was not significantly affected by POMj in either population [17]. Therefore, in response to unilateral eccentric exercise, these authors concluded that POMj supplementation can alleviate exercise-induced soreness of the arm muscles, but not leg muscles, with this beneficial effect more likely to occur in resistance training individuals. Conversely, POMj supplementation has been reported to lower the perception of muscle soreness (i.e., at 48h) in knee extensors, but not the elbow flexors, in elite weightlifters completing whole body resistance exercise [18]. The authors of this study ascribed the absence of a lower soreness perception in elbow flexors after POMj supplementation to the lower soreness provoked by the weightlifting exercises in the arms compared to the legs. Accordingly, the lower muscle pain in the upper compared to the lower

body musculature likely lowered the scope for a POMj-mediated attenuation in muscle soreness in the former compared to the latter. Therefore, it appears that the blunting of muscle soreness post POMj supplementation might be linked to the degree of soreness evoked by a given exercise task.

In addition to inter-study differences in limb-specific muscle soreness responses post POMj supplementation, the studies of Ammar et al. [18] and Trombold et al. [17] yielded contrasting results on the effects of POMj on muscle soreness of the same muscle group (knee extensors) in response to whole body [18] or unilateral [17] resistance exercise. The blunting in knee extensor muscle soreness in the study by Ammar et al. [18], but not Trombold et al. [17], might be linked to the higher polyphenol content of the POMj administered by Ammar et al. [18] (2.56g/500mL vs. 650 mg/480mL, respectively). Alternatively, or in conjunction with the different polyphenol doses administered, the disparate effects of POMj supplementation in these studies could be a function of differences the muscle mass engaged (large muscle mass exercise vs. one limb knee extensor) or the exercise tasks completed (combination of eccentric and concentric vs. eccentric only exercises) in Ammar et al. [18] compared to Trombold et al. [17], respectively. Therefore, the potential for POM supplementation to blunt muscle soreness appears to be positively related to the dose of polyphenols administered and the volume of muscle mass engaged. Thus, dietary supplementation with POM containing a sufficient dose of polyphenols could be an effective treatment to improve the recovery of muscles strength and weakness which might result in a lower fatigue perception and higher performance in the subsequent training session [22, 24].

3.4 Effect of POM supplementation on muscle damage responses

The mechanisms that underpin muscle damage are believed to involve both mechanical and metabolic processes [46]. Since, mechanical and metabolic demands on the skeletal muscles are influenced by the nature of physical activity, it was suggested that the magnitude and the level of muscle damage are affected by the mode, intensity and duration of exercise [47]. To date, two general phases have been proposed to describe the damage responses during and following physical exercise. The first phase is initiated during exercise and involves mechanical and metabolic responses which are collectively referred to as primary or acute damage [47], while the second phase is associated with an ensuing inflammatory response which develops following exercise (i.e., days to weeks) and is termed delayed damage [48]. Of the 11 studies conducted to date, three studies have investigated the effect of POM supplementation on exercise-induced muscle damage with two studies assessing both acute (3 min to 2h) and delayed (1 to 4 days) responses to strength exercises [18, 25] and one study assessing only the delayed effect of POM following an endurance training session [26].

3.4.1 Effect on acute muscle damage responses

In recreationally-active males (Table 2) Trombold et al. [25] showed that, compared to PLA, POMe supplementation had no effect on muscle damage markers 2 hours post unilateral eccentric exercise. Indeed, in response to 2 sets of 20 maximal eccentric elbow flexion repetitions, creatine kinase (CK) was increased to a similar extent in both the PLA and POMe conditions with no change in myoglobin (Mb) 2 hours post exercise. In contrast, using Olympic weightlifting exercises (Table 2), Ammar et al. [18] reported a blunting in muscle damage following whole body resistance exercise in well-trained subjects after POMj supplementation. Specifically, consumption of POMj attenuated the acute increase of CK (-8.75%) and lactate dehydrogenase (LDH) (-1.64%), and blunted the increase of aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP) compared to the PLA condition. Similar to the muscle soreness results, these conflicting results could: (i) be explained by the lower volume of muscle mass engaged, and by extension experiencing damage, in the study of Trombold et al. [25], (ii) confirm that the magnitude of muscle damage responses (and therefore scope for recovery) are affected by the nature of exercise [47], and (iii) reflect an attenuated acute beneficial effect of POM on muscle damage following unilateral exercise.

3.4.2 Effect on delayed muscle damage responses

Similar to the results observed 2h post exercise, Trombold et al. [25] did not observe a beneficial effect of POMj on the delayed damage responses 1-4 days following eccentric unilateral elbow exercise. Indeed at 24h, 72h and 96h post-exercise, no differences in CK and Mb were observed in untrained subjects between the POMe and PLA conditions. These findings were corroborated in trained endurance athletes by Fuster-Munoz et al. [26] who showed that consumption of 200mL of POMj did not affect ASAT and alanine aminotransferase (ALAT) responses during a 3 week training program (Table 2). The authors concluded that the 3week intervention was not sufficient to elicit a blunting in ASAT and ALAT in trained endurance athletes after POMj supplementation and suggested that an extended supplementation period (i.e., intervention period > 3weeks) could result in significant differences between the POMj and PLA groups [26]. However, POMj supplementation had a different effect in well-trained resistance subjects [18]. Indeed, the consumption of POMj 48h before and during the training session accelerated muscle damage recovery 48h post a weightlifting training session by expediting the recovery kinetics of CK (11.43%), LDH (5.08%) and ASAT (4.94%). These results indicated that 48h POMj supplementation can be sufficient to restore muscle damage to baseline levels following an intense strength training session (Table 2). Therefore, a natural POMj with high polyphenol concentration (i.e., 2.56g/500ml) could be a practical and potent treatment to alleviate muscle damage following intense physical exercise, particularly in resistance training individuals.

3.5 Effect of POM supplementation on oxidative stress responses

Oxidative stress reflects an imbalance between pro-oxidant and antioxidant status with the former outweighing the latter [49]. Strenuous exercise or intensified training has been shown to elicit acute oxidative stress during exercise and to exhibit a delayed recovery of oxidative stress biomarkers (lipid peroxidation and enzymatic antioxidant) following exercise cessation [50]. It is well accepted that exercise provokes the development of oxidative stress by enhancing ROS production via increased phospholipase A2 (PLA2), NADPH oxidase and xanthine oxidase (XO) activities [50]. It is recognized that increased ROS exposure can contribute to fatigue during exercise via the oxidation of critical redox-sensitive sites within skeletal muscle [50] and the resulting structural damage to lipids, protein and DNA oxidation. However, recent evidence suggests that ROS are also integral to the adaptive responses of muscle fibers to exercise stress via the activation of transcription pathways that regulate gene and protein expression within skeletal muscle [50]. Despite the high antioxidant capacity of POM (i.e., rich in polyphenols such as anthocyanins, flavonols, and certain ellagitannins such as punicalagin [51]) and its resultant potential to mitigate exercise-induced oxidative stress, few studies (n=5) have assessed the effects of POM on post-exercise oxidative stress [19, 26, 27, 34, 36].

3.5.1 Effect on acute oxidative stress responses

To date, four studies have examined the effect of POM supplementation on oxidative stress biomarkers immediately following physical exercise [19, 27, 34, 36]. Specifically, these studies aimed to evaluate the efficacy of POMj consumption to improve the immediate antioxidant responses to exhaustive exercise in young healthy males [27, 34, 36] and in elite weightlifters [19]. Mazani et al. [27], Naghizadeh-Baghi et al. [36] and Ammar et al. [19] reported that consumption of POMj prior to exercise (240mL/day for 14 days [27, 36] and 750ml/day for 2 days [19]) enhanced the activity of key antioxidant enzymes and attenuated lipid peroxidation immediately after treadmill running (70% maximal heart rate), ultra-endurance exercise and a weightlifting training session. While lipid peroxidation markers and the activity of enzymatic antioxidants were increased at post exercise in both the POMj and PLA groups, the pre-post exercise change was higher for enzymatic (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT)) and non-enzymatic antioxidants (e.g., uric acid (UA) and total bilirubin (Tbil)) and lower for malondialdehyde (MDA) in the POMj condition compared to the PLA condition [19, 27, 36]. These observations support the use of POMj consumption to enhance antioxidant status in humans completing intense exercise [52]. Indeed, these findings are consistent with those of Tsang et al. [34] who showed that one week of POMj consumption (500 mL/day containing 1.69g total phenolics/L) significantly lowered urinary lipid peroxidation levels in the POMj group immediately after 30 min of treadmill running (50% Wmax). Consistent with these findings, previous studies in sedentary subjects reported the effectiveness of POMj supplementation to neutralize ROS [53, 54]. Collectively, the existing literature suggests that POMj supplementation has the potential to blunt exercise-induced oxidative stress.

436

437 ***3.5.2 Effect on delayed oxidative stress responses***

438 The effect of POMj consumption on the delayed oxidative stress response following exercise has only been investigated
439 in the studies of Fuster-Munoz et al. [26] and Ammar et al. [19], which recruited adult endurance and resistance trained
440 males, respectively. In endurance trained males, 22days of POMj supplementation attenuated protein carbonyl (PC) and
441 MDA levels such that these biomarkers were only increased following endurance training sessions in the PLA group
442 (1.1 vs. 1.8 nmol/mg and 14.1 vs. 10.9 nmol/g protein, respectively for PC and MDA), suggestive of a reduction in
443 oxidative stress during the aerobic training session after POMj supplementation [26]. Similarly, resistance trained males
444 exhibited a delayed effect of POMj in response to a weightlifting training session. Indeed, 48h following a resistance
445 exercise session, Ammar et al. [19] reported expedited recovery kinetics of MDA (5.63%) and the antioxidant enzymes,
446 CAT (8.94%) and GPX (10.21%) markers with POMj compared to PLA supplementation. Therefore, POMj
447 supplementation appears to be effective at blunting oxidative stress biomarkers following both endurance and resistance
448 exercise sessions. Consistent with these findings, previous studies in healthy non-active subjects showed that 15 days of
449 POMj consumption increased levels of reduced glutathione (22.6%) and lowered levels of MDA (24.4%) and protein
450 carbonyls (17.7%) even one week after POMj administration has terminated [52]. Collectively, the existing literature
451 suggests that, even after ceasing POMj consumption, some of its beneficial effects on antioxidant status prevail for at
452 least a few days. Although the mechanisms for these delayed effects have not been resolved, they might be linked to a
453 protracted radical scavenging, antioxidant recycling and modulation of antioxidant enzymatic activity [55].

454

455 **3.6 Effect of POM supplementation on inflammatory responses**

456 Intense physical exercise, has been shown provoke a rapid and pronounced local inflammatory response (i.e., invasion
457 of muscle by inflammatory cells). Thereafter, a systemic inflammatory response, known as acute-phase response [56],
458 becomes manifest that can persist for days to weeks [49]. The white blood cells (WBC) are the major cellular mediators
459 of inflammation [57]. The increased prevalence of white blood cells after intense exercise [58] is believed to be mainly due
460 to the rise of neutrophils and monocyte/macrophage influx as determined by the expression of leukocyte adhesion
461 molecules [57]. Additionally, the secretion of pro-inflammatory cytokines, such as TNF-α and interleukin-1 beta (IL-1β),
462 and the inflammation responsive cytokine, IL-6, by the endothelial cells is believed to mediate exercise-induced
463 inflammatory process [56]. Moreover, during physically demanding exercise tasks, high-sensitivity C-reactive protein
464 (hs-CRP), ceruloplasmin and matrix metalloproteinase (MMPs) have previously been classified as biomarkers of
465 inflammation. Indeed, the contraction of skeletal muscle after intense physical activity has been shown to stimulate the
466 local production of MMPs [59], which play a physiological role in muscle regeneration [60] and adaptation [61] to exercise

training. Of the several MMPs, previous studies have shown that MMP2 (gelatinase A) and MMP9 (gelatinase B) play critical roles in remodeling and regenerating skeletal muscle following exercise [62]. Therefore in this section we will focus on the main results of studies (n= 4) which have investigated the effect of POMj supplementation on the acute [18, 25, 27] and delayed [18, 25, 26] responses of inflammatory markers (i.e., WBC, IL, CRP or hs-CRP, MM2 and MM9) following intensive exercises (Table 2).

3.6.1 Effect on acute inflammatory responses

In untrained subjects, Trombold et al. [25] observed no change in IL-6 and hs-CRP responses in either the PLA or POMj group immediately following exercise. In this study, the absence of inflammatory responses following unilateral eccentric exercise could be explained by the small volume of muscle mass recruited. Indeed, when a similar type of exercise has been completed with a larger muscle mass recruited, previous studies have found increases in systemic IL-6 and hs-CRP [63] and local inflammation [64] post exercise. Consistent with this interpretation, Mazani et al. [27] showed a significant increase in inflammatory markers following exhaustive running exercise with higher pre to post exercise changes in MMP2, MMP9 and hs-CRP in the PLA group compared to a group receiving 14 days of POMj supplementation. These results indicate that regular intake of POMj prior to exercise significantly blunts inflammatory responses before and after exhaustive exercise. The acute anti-inflammatory effect of POMj observed in sedentary subjects in the study of Mazani et al. [27] has been recently confirmed by Ammar et al. [18] using resistance trained subjects. Indeed, the consumption of POMj during the 48h (1500ml) and the last 1h (500ml) prior to a weightlifting training session, which recruited a large muscle mass, was found to attenuate the increase in hs-CRP post exercise. Collectively, these findings support the anti-inflammatory properties of polyphenol-rich POMj supplementation previously reported in sedentary healthy subjects and patient populations [9, 14, 15, 62] and suggest that the beneficial effect of POM is influenced by the volume of skeletal muscle mass recruited during exercise. Although the underlying mechanisms of the anti-inflammatory effects of POM supplementation are not entirely clear, various explanations have been proposed. For example, Ammar et al. [18] suggested that the lower post-exercise level of hs-CRP following POMj supplementation could be due to the inhibition of some inflammatory markers such as NF-κB, TNFα and COX-2. Additionally, given that the inhibition of MMPs by tumour necrosis factor (TNF) has been reported to be dependent on reducing ROS production [65], and since polyphenolic-compounds present in POM have been shown to confer antioxidant properties that inhibit ROS production [53], blunted ROS production following POM supplementation might contribute to its anti-inflammatory effects [27].

3.6.2 Effect on delayed inflammatory responses

In endurance trained athletes, Fuster-Munoz et al. [26] reported that the levels of sE-selectin and hs-CRP over 22 days following aerobic training sessions was not measurably impacted by the consumption of POMj. Similarly, in resistance trained athletes, Ammar et al. [18] observed no effect of POMj on the recovery kinetics of hs-CRP and WBC levels 48h following a weightlifting training session. However, given that hs-CRP better reflects endothelial dysfunction and vascular inflammation than muscular function [26], future studies would benefit from evaluating the effect of POM supplementation on the profiles of cytokines such as TNF or IL6 which better relate to exercise performance [66].

3.7 Effect of POMj/e on cardiovascular parameters

It is well established that the demand for oxygen and metabolic substrates increases in the contracting skeletal muscles during physical exercise. To meet these elevated demands, blood flow to working musculature is increased during exercise [37]. Nitric oxide (NO) production has been shown to be an import contributor to exercise-induced skeletal muscle hyperemia [67]. Polyphenols have also been reported to improve cardiovascular function during stressful situations [68, 69]. Given that polyphenol-rich POMj has been reported to protect NO from oxidative scavenging and to enhance the biological actions of NO [38], the beneficial effect of POMj supplementation on cardiovascular function might be NO-mediated. Although previous studies have investigated the effect of POM supplementation on heart rate (HR), blood flow, vessel dilation and cardiovascular function in sedentary subjects [6, 7, 12, 53, 67], a limited numbers of studies have investigated the effect of POM supplementation on these parameters during exercise and to what extent this might contribute to a potential ergogenic effects of POM supplementation. This section will focus on the main findings of studies (n= 4) that have investigated how the consumption of POM impacts cardiovascular responses immediately [18, 32, 34, 35] and up to 48h [18] following running or strength exercise (Table 2).

3.7.1 Effect on acute cardiovascular responses

To date, two studies have investigated the effect of POM on blood flow and vessel diameter responses immediately following physical exercise [28, 35]. In these studies the consumption of 1000 mg of POMe (2 × 500mg capsules) 30min prior to exercise was reported to increase vessel diameter (VD) and blood flow (BF) immediately, and up to 30 min, after exhaustive exercise compared to a placebo condition. Indeed, POMe supplementation has been reported to result in a larger VD (0.42±0.07 vs. 0.39±0.07 cm) and higher BF (40.6±24.8 vs.29.6±24.9 ml/min) 30min post-POMe ingestion and 30min post 3 treadmill runs to exhaustion (at 90,100, and 110% PV) [28]. Similarly, POMe ingestion lead to a larger VD immediately following leg press and bench press exercise at 80% 1RM continued to fatigue (mean difference = 0.042 cm for leg press and 0.029 cm for bench press) and 30 min post leg press (mean difference = 0.029) [35]. This beneficial effect of POMe was also observed following a RSA test with higher BF and VD observed

529 respectively immediately and 30 min post exercise following POMe ingestion [35]. Given that NO production is an
530 important contributor to vasodilatation [37], and that polyphenols have been shown to phosphorylate and thereby activate
531 endothelial nitric oxide synthase (eNOS) [38, 70], the results of Roelofset et al. [28] could be explained by the high content
532 of polyphenols in the POMe supplement (3500 µmol/L). Moreover, the protective role of POM against ROS-mediated
533 NO scavenging [38] could also explain the enhanced vasodilation following exercise.
534 Assuming POM supplementation increases blood flow during exercise, the associated increase in nutrient delivery to,
535 and efflux of noxious metabolic by-products from, skeletal muscle [37, 67] might contribute to ergogenic effect of POM
536 supplementation during exercise and the enhanced post-exercise recovery [17,18, 25, 32]. In addition to effects on blood
537 flow and vessel diameter [32, 35], POM has been reported to lower blood pressure and HR during physical exercise [18, 34].
538 Indeed, daily consumption of POMj (500ml, 1.69g total phenolic/l) for one week prior to exercise [34] was shown to
539 lower the systolic blood pressure (SBP) and the diastolic blood pressure (DBP) before and immediately after 30min of
540 treadmill exercise (50%Wmax). Likewise, the consumption of POMj (500ml) 1h before the training session was shown
541 to attenuate the acute increase of SBP (-4.46%) and HR (-1.81%) immediately (i.e., 3min) after intense weightlifting
542 exercise [18]. The reduction in post-exercise blood pressure and HR with POMj, if also observed during exercise, implies
543 that POMj supplementation might improve performance and lower the perception of fatigue [17, 18, 25] by improving
544 aspects of cardiovascular function.
545
546 ***3.7.2 Effect on delayed cardiovascular responses***
547 The effect of POM on the delayed recovery of cardiovascular responses following exercise is currently unclear. Indeed,
548 only one study has examined SBP and HR responses 48h post exercise after POMj supplementation [18]. To our
549 knowledge, the delayed responses of blood flow, vessel diameters and DBP have yet to be assessed. The consumption
550 of POMj prior to an intensive weightlifting training session improved the recovery kinetics of SBP 48h post-exercise in
551 elite weightlifters [18]. Given that the reduction of SBP following POMj has been linked to a reduction in the
552 cortisol/cortisone ratio [34], the beneficial effect of POMj on SBP during exercise could be the result of 11β-HSD1
553 inhibition [34]. However, further studies are necessary to resolve the mechanisms for the improved cardiovascular
554 function following POM supplementation.
555
556 **4 Discussion**
557 This systematic review evaluated the existing literature assessing the effect of POM supplementation on physical
558 performance, muscle soreness and physiological responses during and following different exercise sessions. Based on
559 the studies assessed in this review, POM supplementation appears to hold potential as a nutritional aid to enhance

performance during endurance [28, 35] and strength [17, 18, 25, 33] exercise, and to expedite enhanced post exercise recovery of skeletal muscle function [17, 25, 33]. These improvements in exercise performance and recovery have been linked to an attenuation of muscle damage [18] ~~following weightlifting exercise~~; lowered oxidative stress [19, 26, 27, 34, 36] ~~following exhaustive strength exercise, treadmill running and ultra-endurance exercise~~ and inflammation [18, 27] ~~during exhaustive running exercise~~; and enhanced cardiovascular function [18, 28, 34, 36] ~~during strength and treadmill running exercise~~. This review has potential implications for improving the use of POM supplementation by athletes, nutritionists and coaches to enhance exercise performance and post-exercise recovery.

Dietary supplementation with POM has shown promising potential to enhance physiological responses in sedentary individuals and patient populations under conditions of physiological strain [7-11]. Since physical exercise is a potent and multifaceted physiological stressor, as evidenced by an increase in muscle damage, oxidative stress, inflammation and cardiovascular strain [20-25], a number of recent studies have examined the potential for POM supplementation to enhance exercise performance and post exercise recovery. When the existing literature was systematically reviewed in the current study, POM was shown to enhance performance and alleviate muscle fatigue and soreness using intermittent running [28, 35] and strength exercises [17, 18, 25, 33]; to blunt muscle damage following weightlifting exercises [18]; to promote an antioxidant effect following exhaustive strength exercises [19] treadmill running [27, 34] and ultra-endurance exercise [26, 36]; to confer an anti-inflammatory effect during exhaustive running exercise [27]; and to promote beneficial effects on the cardiovascular system during strength [18, 35] and treadmill running exercise [28, 34, 35].

The positive effects of POM supplementation are likely linked to its high content of polyphenols. Previous studies investigating the effect of polyphenol supplementation have reported increases in blood flow, vessel dilation [67] and endothelial function [71]. This potential for enhanced vasodilation following polyphenol supplementation could improve nutrient delivery to and promote the efflux of noxious metabolic by-products from skeletal muscle which might have implications for accelerating muscle recovery [28, 37]. In addition to enhanced cardiovascular function, polyphenol supplementation protects against the development oxidative stress [72] and inflammation [71]. Accordingly, POM supplementation might aid exercise performance and recovery by enhancing cardiovascular function and mitigating oxidative stress and inflammation. In particular, the ergogenic and recuperative effects of POM supplementation might be linked to the scavenging of free radicals [73]. Specifically, polyphenols can attenuate oxidative damage through the rapid donation of an electronto a free radical from –OH groups [74, 75]. Therefore, polyphenols are capable of reducing, stabilizing and inactivating free radicals species, thereby inhibiting lipid peroxidation and preventing against atherosclerosis and long-lasting Ca2+ release events [76, 77]. Furthermore, modulating antioxidant enzymes and chelating

metal ions (Fe^{2+}, Cu^{2+}; involved in free radical production), and the associated blunting of free radical production, are reported to be among the most important mechanisms mediating the protective effect of polyphenol-rich foods [78, 79]. Other possible mechanisms by which polyphenol-rich supplements exert their beneficial effects are thought to include the inhibition of leukocyte immobilization and xanthine oxidase activity [74]; enhanced endothelial and mitochondrial function [80]; and the recycling of antioxidant and reducing agents to boost antioxidant defense systems (e.g., vitamin E and C) [73, 79].

The potential significance of polyphenols in mediating the positive physiological effects of POM supplementation is supported by observations that the variable polyphenol content of the POM supplements administered and the daily dose of POM consumed (presented in tables 1 and 2) might influence the inter-study disparity in the efficacy of POM supplementation. For example, the consumption of natural POMj containing 2.56g total polyphenols /0.5 L three times per day (3 × 250mL) during the 48h period prior exercise has been reported to confer anti-damaging effects (i.e., acute and delayed) in responses to intense weightlifting exercise [18]. Conversely, the consumption of a commercially produced POM (Wonderful bottle, Los Angeles, CA) that contained only 0.65 g total polyphenols/0.5 L two times per day (2 × 250ml), did not influence muscle damage following unilateral eccentric exercise [25]. These results imply that 750mL of polyphenol-rich POMj (> 0.7g/0.5 L) could be an important dosing threshold for POMj supplementation to confer anti-damaging effects during exercise. Similarly, the nature of exercise was also identified as an important mediator of the positive physiological effects of POM. Indeed, based on the existing evidence it appears that the potential benefits of POM supplementation are attenuated following eccentric exercise. This attenuation could be explained by the harmful biological responses following eccentric contraction compared to a combined or dynamic strength exercise. Indeed, although the energy cost is lower for eccentric contractions compared with concentric ones, for the same power output, the former can cause a large degree of muscle, cellular and oxidative damage [81-83] due to the increased generation of tension as muscle lengthens, resulting in a higher load per fibre ratio [84]. Nevertheless, it should be acknowledged that many other factors could underlie the disparate inter-study results including the training status of the subjects (untrained vs, trained), the type of exercise assessed (unilateral eccentric, weightlifting, running treadmill), and the duration of the investigation (30min, 48h, > 1 one week). Therefore, standardizing these factors in future studies is important to resolve the potential efficacy of POM supplementation to enhance exercise performance, physiology and recovery and to optimize recommendations for best practice with POM supplementation .

Although consumption of polyphenol-rich beverages (e.g., polyphenols specific to POM, including flavonols, ellagitannins, and anthocyanins) can modulate oxidative stress, muscle damage, inflammation and improve

cardiovascular function and exercise recovery and performance [18,19,34 ,67, 68, 73], it has been reported that a long term (8 weeks) consumption of the polyphenol, trans-resveratrol (250 mg/day), can blunt the beneficial effects of exercise on the lowering blood pressure, and blood concentrations of several cardiovascular risk factors in elderly men [85]. While the exact mechanism mediating the absence of a potential complementary synergy between exercise and resveratrol was not addressed in this study [85], the authors suggested that enhanced antioxidant defense in the resveratrol group may have retarded the exercise-induced increase in maximal oxygen uptake by abrogating ROS [86, 87] which are now recognized as important signaling molecules that contribute to the adaptations to exercise training [88]. Taken together, the results of the available studies indicate that, while the powerful antioxidant effect of polyphenols can blunt redox perturbation and muscle damage, and accelerate the recovery of skeletal muscle force production post strenuous exercise in the short term, the long term effects of continuous polyphenol supplementation and the accompanying antioxidant effect could disrupt some of the physiological adaptations elicited by a training program. These findings suggest a balance exists between the beneficial and undesirable effects of polyphenol supplementation which requires consideration in future research. Specifically, it is unclear whether the polyphenol blend that comprises POM promotes a similar blunting in exercise training adaptations as the polyphenol, resveratrol and what supplementation strategy with POM might optimize the balance between promoting recovery from specific training sessions without attenuating the exercise-induced redox signaling that provokes the physiological adaptations to exercise training. This requires addressing in future studies to optimize POMj supplementation guidelines.

4.1 Comparison between the effect of POM and other nutritional interventions during exercise

It has been well established in sedentary individuals that POM possesses a higher antioxidant capacity compared to other supplement such as red wine, blueberry juice, cranberry juice, orange juice, green tea and wine vinegars [16, 51, 89]. Similarly, POM supplementation has shown potential to enhance muscle performance as evidenced by reduced DOMS, muscle damage, oxidative stress and inflammation, and improved cardiovascular responses during and following exercise [17-19, 25-27, 32]. Nevertheless, it should be acknowledged that other dietary supplementation strategies have also exhibited similar ergogenic and protective effects during exercise. Conversely, supplementation with vitamin C or E does not influence strength performance and soreness post exercise [90]. Although dietary supplementation with acombination of tocopherols, flavonoids (i.e., Hesperetin and quercetin), selenium or docosahexaenoic acid [63], and the mixture of ascorbic acid, α-tocopherol, and selenium [91],can attenuate systematic inflammation (CRP and IL-6) and oxidative stress after eccentric exercise, the effect of this nutrient combination on strength performance and DOMS has yet to be assessed. On the other hand, polyphenols specific to POM, including flavonols, ellagitannins, and anthocyanins have demonstrated a positive effect on endothelial-dependent vasodilation, and importantly, this effect is

greater than achieved with other fruits containing a different mix of polyphenols [92]. Polyphenol supplementation from tart cherries has been shown to improve strength recovery following a bout of eccentric elbow flexion contractions (i.e., lower strength loss and pain [93]), completion of a marathon (i.e., faster recovery of isometric strength [94]) and prolonged, intermittent shuttle exercise (i.e., faster recovery of performance indices [95]). The enhanced recovery of muscle function after ingesting tart cherries was accompanied by increased total antioxidant capacity, and lower lipid peroxidation (TBARS) and attenuated inflammation markers (IL-6 and CRP) [94, 95]. However, no other indices of muscle damage (CK and LDH), or oxidative stress (LOOH and PC) were different between the PLA and the cherry juice groups [94, 95]. Additionally, consumption of multi-ingredient performance supplements (MIPS) 30min prior to exercise for eight weeks has been shown to improve bench press strength, lean body mass and quadriceps muscle thickness without impacting leg press strength [96]. Collectively, these results suggest that POM supplementation could be an effective treatment to improve performance, muscle recovery and to reduce weakness and damage in responses to physical exercise. It also appears that POM supplementation compares favorably with other polyphenol-rich foods with regard to enhancing exercise performance and recovery, but further research is required to directly compare the efficacy of POM to enhance exercise performance and recovery compare to other polyphenol-rich foods.

4.2 Methodological considerations

It is important to stress that, while the polyphenol content of POM is positively associated with its protective effect against damage during exercise and with exercise performance [18,19], POM does not exhibit a uniform polyphenol content throughout the fruit [97]. Indeed, higher levels of polyphenols are present in the inner and outer peels than in the seeds [7]. These observations underscore the importance of the juice manufacturing method and suggest that POMj/e which contains a mixture of seeds and peels [18, 19, 26] is more likely to be beneficial for enhancing physiological and functional responses during and following physical exercise. Another important consideration that appears to influence the efficacy of POM supplementation is the supplementation regime employed. Indeed, it has been reported that POM consumed 30min prior to exercise improves intermittent capacity without impacting high-intensity anaerobic performance [32], while POM consumed 60min prior exercise was able to improve high-intensity anaerobic performance (i.e., weightlifting exercises) [18]. Therefore, it would appear advantageous to consume POM supplements at least 60min prior to intensive anaerobic exercise to provide sufficient time to elicit a potential ergogenic effect on both aerobic and anaerobic performance. However, to optimize supplementation guidelines, the dose-response and pharmacokinetics of POM supplementation must be elucidated. Another important consideration for studies wishing to assess the efficacy of POM supplementation is that the beneficial effect of POM supplementation can persist for up to three weeks after consumption [52]. Accordingly, when a crossover experimental study design is adopted, the wash out period between supplements should be greater than 3weeks to avoid any potential confounding influence of the POM supplementation,

683 if administered first, on the second supplementation arm of the study. Concerning the selection of biomarkers, it should
684 be acknowledged that since exercise has been shown to provoke muscle damage, inflammation and oxidative
685 modifications to several biological components [20-24, 98] and since at least two or more biomarkers has been
686 recommended to accurately infer oxidative, muscle or inflammatory damage [99, 100], future studies should use multiple
687 related biomarkers (e.g., at least: MDA and PC to measure oxidative stress; CK and LDH to measure muscle damage
688 and hs-CRP, IL-6 and TNF to detect inflammation) to confirm the potential positive effects of POM supplementation on
689 blunting exercise-induced oxidative stress, muscle damage or inflammation. Moreover, given that the effects of
690 polyphenol derivatives (flavonols, ellagitannins, anthocyanins, resveratrol) on the biological response and adaptations to
691 exercise training is controversial [18, 19, 67, 68, 85], it is also recommended that future studies investigating the potential
692 synergistic or antagonistic link between exercise adaptations and POM supplementation present the exact composition
693 of polyphenols in POM. This information could help elucidate the mechanisms for the synergistic or antagonistic effects
694 of acute and long term POMj supplementation of exercise performance, recovery and adaptation.
695

696 **5 Conclusion**

697 The review indicates that POM has the potential to enhance endurance and strength performance and to expedite post-
698 exercise recovery by conferring antioxidant and anti-inflammatory effects and improving cardiovascular responses
699 during and following exercise. However, positive effects of POM supplementation are more likely when POMj contains
700 > 0.7g total polyphenols/0.5 L, when large muscle mass exercise is engaged and when POMj is ingested at least 60 min
701 prior to exercise. Therefore, the inclusion (750ml/day) of polyphenol-rich POM in the diet of active people prior
702 (60min) and after exercise (during 48h) could be beneficial for their physical performance and muscle recovery during
703 and following the physical tasks. However, further research is required to assess how chronic POM supplementation
704 impacts the physiological and performance adaptations to exercise training to help optimize POM supplementation
705 guidelines for a range of exercise settings.

706 **Declarations**

707 ***Authors' contributions:*** AA: drafting the article, SJB: revise critically the article, HC: revise critically the article. AH:
708 revise critically the article, NS: revise and give final approval. All authors have read and approved the final version of
709 the manuscript, and agree with the order of presentation of the authors

710 ***Competing interests:*** On behalf of all authors, the corresponding author states that there is no conflict of interest

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Table 1: Effect Of POM on physical performance and fatigue and muscle soreness responses following exercise

Study design							POM effect on:		
Authors	**Subjects**	**Dosage**	**Dura-tion**	**Design**	**Phenolc ontent**	**Exerciseprotocol**	**Performance**	**Fatigue/Soreness**	
								Aute	**Delayed**
Trombold et al. [25]	16 recreational ly active males	2 × 0.5L/day POMj, 12h interval	9 days (4days prior, 5days after ex)	Double-blind, randomized, PLA-controlled crossover design (14 days washout)	0.66g/ 0.5L	Unilateral eccentric elbow flexion (2 × 20)	↑ elbow flexor (i.e., strength) at 48h and 72h post-ex	DOMS: ↓ elbow flexor at 2h post-ex	DOMS: ↔ elbow flexor at 24 to 96 h post-ex
Trombold et al. [17]	17 resistance trained males	2×0.25L/day POMj, 12h interval	15 days (7 days prior, 8 days after ex)	Double-blind, Randomized, PLA-controlled crossover design (14 days washout)	0.66g/ 0.5L	Unilateral eccentric: elbow-flexion(2 × 20); knee-extension (6 × 10)	↑ elbow flexor (i.e., strength) up to 168h post-exercise; ↔ Knee extensor	DOMS: ↔ elbow flexor and knee extensor at 2h post-ex	DOMS: ↓ elbow flexor at 48h and 72h post-ex; ↔ Knee extensor
Machin et al. [33]	45 recreational ly active males	1 × or 2 × 0.5L/dayPOMj	8 days (4days prior, 4days after ex)	Double-blind, randomized, PLA-controlled, 3 arms design	0.65g / 0.5L	10 sets(× 2min) downhill running + 40 bilateral eccentric elbow flexion	↑ strength recovery of both arm and leg muscles		
Trexler et al. [32]	19 (10 M, 9 F) highly active	1000 mg, POMe	30 min pre-ex	Double-blind, randomized, PLA-controlled crossover design (7 days washout)		3 treadmill runs to exhaustion (TTE) at 90, 100 and 110% PV	↑ sub-maximal aerobic performance (TTE at 90 and 100%); ↔ anaerobic running capacity, critical velocity, TTE at 110%	↔ visual analog pain scale ; ↑ vitality scale	
Ammar et al. [18]	9 elite weight-lifters	0.5L 1h prior ex + 1.5L 48h post-ex (3×0.25L/d, 8h interval)	1h pre-ex and 2 days post-ex	Double-blind, non-randomized, PLA-controlled crossover design (48h washout)	2.56g/ 0.5L	Intense weightlifting training session	↑ total and maximal load lifted	↓ perception of muscle fatigue values (RPE)	DOMS: ↓ Knee extensor; ↔ elbow flexor at 48 h post-ex
Roelofset al. [35]	19 (8M, 11F) recreational ly resistance-trained	1000 mg, POMe	30min prior exercise	Double-blind, randomized, PLA-controlled crossover design (7days washout)	3500 µmol/L	high-intensity exercises (i.e., RSA, RTF at 80% on bench and leg press)	↑ average and peak power only in sprint 5 of the RSA ↔ RTF on bench and leg press		

POMj: Pomegranate juice, POMe: Pomegranate extract, PLA: Placebo, M: male, F: female, ex: exercise, TTE: treadmill runs to exhaustion, PV: peak velocity, RSA: repeated sprint ability, RTF: repetitions to fatigue, RPE: rating of perceived exertion, DOMS: delayed onset muscle soreness.

940 **Table 2:** Effect of POM on muscle damage, oxidative stress, inflammatory and cardiovascular responses following exercise

Study design							POM effect on:							
							Muscle damage		Oxidative stress		Inflammation		Cardiovascular	
Authors	**Subjects**	**Dosage**	**Duration**	**Design**	**Phenolcontent**	**Exerciseprotocol**	**Acute**	**Delayed**	**Acute**	**Delayed**	**Acute**	**Delayed**	**Acute**	**Delayed**
Trom-bold et al. [25]	16 recreationally active males	2 × 0.5L/d POMj	9days (4 pre and 5 post-ex)	Double-blind, randomized, PLA-controlled crossover ,(14 days washout)	0.66g/0.5L POMj	Unilateral eccentric elbow flexion (2×20)	↔ CK, MB at 2h post-ex	↔ CK, MB after 1 to 4 days			↔ IL-6, hs-CRP, 2h post-ex	↔ hs-CRP, IL-6, after 1 to 4 days		
Tsang et al. [34]	20 recreationally active	0.5L/d POMj	1 week pre-ex	Double-blind, randomized PLA- controlled, 2-arms	1.69 g total pheno-lics/L	30 min treadmill exercise (50% W_{max})			↓ TBARS 30min after-ex				↓ SBP and DBP 30min after-ex	
Trexler et al. [32]	19 (10 M, 9 F) highly active	2×0.5g/d POMe	30min pre-ex	Double-blind, randomized, PLA-controlled crossover ,(7 days washout)		3 treadmill runs to exhaustion at 90,100, and 110% PV							↑ blood flow and vessel diameters at 30 min post-ex	
Mazani et al. [27]	28 males enduran ce-athletes	0.24L/d POMj	2 weeks, pre-ex	Double-blind, randomized PLA- controlled, 2-arms		treadmill runs at 70% maxHR			↑ GPX, and SOD; ↓ MDA after-ex		↓ MMP2, MMP9, hs-CRP after-ex			
Naghizadeh-Baghi et al. [36]	28 malesen durance-athletes	0.24L/d POMj	2 weeks, pre-ex	Double-blind, randomized PLA- controlled, 2-arms		severe based-endurance activity			↑ ARE, SOD, GPX, TAC; ↓MDA after-ex					

Fuster-Munoz et al. [26]	20 endurance trained males	0.2L/d POMj	21days, pre-ex	Double-blind, randomized, PLA- controlled, 3-arms		1h/d based endurance-training, 3sessions/ week		↔ ASAT and ALAT at day 22		↓MDA, PC at day 22		↔ sE-selectin and CRP at day 22		
Ammar et al. [18]	9 elite weight-lifter	0.5L 1h prior ex + 1.5L 48h post-ex (3×0.25L/d, 8h interval)	1h pre-ex and 2 days post-ex	Double-blind, non randomized, PLA-controlled crossover (48h washout)	2.56g/0.5L POMj	Intense weightlifting training session	↓ CK, LDH, ASAT, at 3 min post-ex	↓ CK, LDH, ASAT at 48h post-ex			↓ hs-CRP 3min post-ex	↔ hs-CRP and WBC at 48h post-ex	↓ SBP and HR 3min post-ex	↓ SBP, ↔ HR at 48h post-ex
Ammar et al. [19]	9 elite weight-lifter	0.5L 1h prior ex + 1.5L 48h post-ex (3×0.25L/day, 8h interval)	1h pre-ex and 2 days post-ex	Double-blind, non randomized, PLA-controlled crossover (48h washout)	2.56g/0.5L POMj	Intense weightlifting training session			↓MDA, ↑GPX, CAT, UA, Tbil, at 3min post-ex	↓MDA, at 48h post-ex				
Roelofset al. [35]	19 (8M, 11F) recratio nally resistance-trained	1000 mg, POMe	30min prior exercise	Double-blind, randomized, PLA-controlled crossover (7-10 days washout)	3500 µmol/L	high-intensity exercises (i.e., RSA, RTF at 80% on bench and leg press)							↑blood flow and vessel diameters, ↔ SPO_2, HR and BP at 0, 30 min post-ex	

POMj: Pomegranate juice, POMe: Pomegranate extract, PLA: Placebo, M: male, F: female, ex: exercise, TTE: treadmill runs to exhaustion, PV: peak velocity, RSA: repeated sprint ability, RTF: repetitions to fatigue, CK: creatine kinase, LDH: lactate deshydrogenase, MB: myoglobin, ASAT : aspartate aminotransferase, TBARS: thiobarbituric acid-reactive substances, MDA: malondialdehyde, PC: protein carbonyl, TAC: total antioxidant capacity, GPX : glutathion peroxidase, SOD: superoxide dismutase, CAT : catalase, UA: uric acid, ARE: arylesterase, hs-CRP: high sensitive c-reactive protein, IL-6: interleukin (6), MMP : matrix metalloproteinases, WBC : white blood cell, HR: heart rate, SBP: Systolic blood pressure , DBP: diastolic blood pressure, SPO2: oxygen saturation .

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953 **Figure 1:** Flowchart of study selection. PEDro = Physiotherapy evidence database scale

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