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ROZPRAWA DOKTORSKA

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Wpływ suplementacji sokiem z owocu granatowca właściwego (*Punica granatum* L.) na zdolność antyoksydacyjną osocza i gospodarkę żelazem u osób trenujących wyczynowo wioślarstwo

W formie spójnego tematycznie cyklu artykułów opublikowanych w czasopismach naukowych

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DOCTORAL DISSERTATION

Alicja Urbaniak

Effect of supplementation with pomegranate fruit juice (*Punica granatum* L.) on antioxidant capacity of plasma and iron metabolism in rowers

In the form of series of articles published in scientific journals

Advisor: Prof. AWF dr hab. Anna Skarpańska-Stejnborn

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SPIS TREŚCI / INDEX

I. AUTOREFERAT W JĘZYKU POLSKIM

Podstawą rozprawy doktorskiej jest cykl publikacji pod zbiorowym tytułem: „Wpływ suplementacji sokiem z owocu granatowca właściwego (Punica granatum L.) na zdolność antyoksydacyjną osocza i gospodarkę żelazem u osób trenujących wyczynowo wioślarstwo". W skład dysertacji wchodzą dwie prace opublikowane w czasopismach o zasięgu międzynarodowym.

1. **A. Urbaniak**, A. Skarpańska-Stejnborn, Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic review, ***Int. J. Vitam. Nutr. Res.*** 1-15 (**2019**) – 5 letni impact factor – 1.251, punktacja ministerstwa – 100 pkt.

2. **A. Urbaniak**, P. Basta, K. Ast, A. Wołoszyn, J. Kuriańska-Wołoszyn, E. Latour, A. Skarpańska-Stejnborn, The impact of supplementation with pomegranate fruit (*Punica granatum* L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers, ***J. Int. Soc. Sport. Nutr.*** 15:35 (**2018**) - 5 letni impact factor – 3.508, punktacja ministerstwa – 100 pkt.

WSTĘP

Celem badań była analiza wpływu podaży soku z owocu granatowca właściwego (*Punica granatum* L.) jako potencjalnie skutecznej, możliwej do osiągnięcia i bezpiecznej terapii w celu ograniczenia szkodliwego wpływu intensywnego wysiłku fizycznego na organizm sportowców wyczynowych.

Intensywny wysiłek fizyczny prowadzi do znacznego obciążenia organizmu sportowca, które może skutkować zaburzeniem wewnętrznej homeostazy, a także negatywnie wpływać na wynik sportowy. Jednym z kluczowych czynników, które mają wpływ na wydolność zawodnika jest metabolizm żelaza (Buratti *et al.*, 2015) (Otto, Montgomery and Richards, 2013). Żelazo bierze udział w wielu procesach fizjologicznych m.in., transporcie tlenu czy też pozyskiwaniu energii (Gozzelino and Arosio, 2016). Zaburzenie homeostazy żelaza może być spowodowane nie tylko niedoborem tego pierwiastka w diecie, ale również stanem zapalnym wywołanym przez intensywny wysiłek fizyczny (Dahlquist *et al.*, 2017). Stan zapalny w organizmie związany jest ze wzmożoną syntezą hepcydyny – hormonu biorącego udział w degradacji ferroportyny, której rolą jest ograniczenie migracji żelaza z jego rezerw komórkowych (wątroby, śledziony) oraz redukcję wchłaniania tego pierwiastka z przewodu pokarmowego (Nemeth, Tuttle, *et al.*, 2004)(Sukumaran *et al.*, 2012). Należy podkreślić, że utrzymywanie się takiego stanu skutkować może ograniczeniem erytropoezy i w konsekwencji prowadzić do rozwoju niedokrwistości (Liu *et al.*, 2011). Z przeprowadzonych do tej pory badań wynika, że pod koniec sezonu sportowego aż u 70% zawodników uprawiających sport wyczynowo (wioślarzy i piłkarzy) zaobserwowano funkcjonalny niedobór żelaza, a u 27% absolutny niedobór tego pierwiastka w surowicy (Reinke *et al.*, 2012).

Suplementacja preparatami bogatymi w żelazo nie zawsze przynosi oczekiwane rezultaty, a jej skutki mogą okazać się szkodliwe dla zdrowia (Clénin *et al.*, 2016)(Ishibashi *et al.*, 2017). W badaniach *in vivo*, które zostały przeprowadzone na zwierzętach laboratoryjnych, zastosowano domięśniową iniekcję z żelaza, która skutkowała wzrostem stężenia tego pierwiastka w mięśniach i osoczu oraz nasileniem stresu oksydacyjnego. W rezultacie u zwierząt tych odnotowano istotnie niższą siłę mięśniową niż w grupie kontrolnej (Reardon and Allen, 2009).

Powysiłkowy wzrost poziomu hepcydyny może być również związany z pojawieniem się tzw. niezwiązanej (wolnej) puli żelaza, która pochodzi z uszkodzonych pod wpływem wysiłku fizycznego erytrocytów (Buratti *et al.*, 2015)(Berzosa *et al.*, 2011). Należy podkreślić, że tzw. niezwiązane żelazo może katalizować reakcje wolnorodnikowe prowadząc do nasilenia hemolizy.

W przeprowadzonych ostatnio badaniach, stwierdzono korzystny wpływ soku z owocu granatowca na zaburzenia spowodowane niedoborem żelaza w organizmie człowieka (Shema-Didi *et al.*, 2013). Rezultaty tych prac przyczyniły się do wzrostu zainteresowania tym owocem jako suplementem diety dla sportowców wyczynowych (Ammar *et al.*, 2018).

Sok z owocu granatowca stanowi bogate źródło polifenoli. Są to przede wszystkim: antocyjany, flawonole i niektóre elagitaniny. Wśród nich szczególnymi właściwościami cechują się antocyjany. Związki te wykazują szereg aktywności biologicznych, wśród których wyróżnić można: właściwości przeciwutleniające (CĂta *et al.*, 2016), stymulujące układ odpornościowy, redukujące stan zapalny (Karlsen *et al.*, 2007), a także wykazujące zdolność chelatowania jonów żelaza, ograniczając w ten sposób zasoby jonów niezwiązanych (Kelsey *et al.*, 2011).

CEL BADAŃ PRZEDSTAWIONYCH W DWÓCH ARTYKUŁACH

Celem badań przedstawionych w artykule przeglądowym pt. „Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic review" było zestawienie i porównanie dotychczasowych wyników związanych z suplementacją preparatami zawierającymi ekstrakt z granatu w grupie sportowców i osób aktywnych fizycznie.

Głównym celem badań omówionych w artykule pt. „The impact of supplementation with pomegranate fruit (*Punica granatum* L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers" było poznanie mechanizmu regulacji gospodarką żelaza w trakcie i po

intensywnych wysiłkach fizycznych oraz rola, jaką mogą w tym procesie odgrywać flawonoidy zawarte w soku z granatu. W szczególności podjęte badania miały na celu uzyskanie odpowiedzi na pytanie: czy suplementacja sokiem z granatu obfitującymi w polifenole przyczyni się u osób poddanych regularnym wysiłkom fizycznym do: ograniczenia stanu zapalnego oraz czy, i w jakim stopniu, zmiany te miały wpływ na wybrane parametry metabolizmu żelaza.

Hipoteza niniejszej pracy doktorskiej oparta została na założeniu, że suplementacja sokiem z owocu granatowca może poprzez wzrost potencjału antyoksydacyjnego przyczynić się do ograniczenia stanu zapalnego indukowanego intensywnym wysiłkiem fizycznym u zawodników wysokokwalifikowanych, uprawiających wioślarstwo. Analizowano również czy, i w jakim stopniu, zmiany te miały wpływ na wybrane parametry metabolizmu żelaza.

PUBLIKACJA 1

METODOLOGIA

Artykuł przeglądowy został napisany według wytycznych opublikowanych przez Pautasso (Pautasso, 2013). Wyniki zostały przedstawione zgodnie ze wskazówkami PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). Artykuły zamieszczone w pracy przeglądowej pozyskano z bazy czasopism Web of Science, gdzie użyto następujących haseł wyszukiwania: "pomegranate supplementation", "pomegranate exercise", "pomegranate sport" lub "Punicagranatum L. supplementation". W pracy uwzględniono również publikacje, które nie wystąpiły w w/w bazie, ale były cytowane w tych artykułach. W celu zapewnienia najlepszej jakości pracy wykluczone zostały artykuły: opublikowane przed rokiem 2000, napisane w innym języku niż angielski, abstrakty konferencyjne, komunikaty oraz inne publikacje przeglądowe.

Wybrane artykuły dotyczyły jedynie prac eksperymentalnych związanych z wpływem suplementacji preparatami opartymi na soku z granatowca, które zostały przeprowadzone na grupie zawodników lub osób aktywnych fizycznie. Wyniki nieopublikowanych badań lub rozpraw naukowych nie zostały uwzględnione w przedstawionym artykule przeglądowym. Uwzględniając powyższe kryteria wyszukiwania, do analizy zakwalifikowano 58 artykułów z bazy danych oraz cztery artykuły pozyskane na podstawie odnośników literaturowych. Po usunięciu duplikatów wybrano 53 publikacje. 18 z nich zostało wykluczonych na podstawie rodzaju artykułu (komunikat, abstrakt konferencyjny itp.), a 24 nie dotyczyło badań eksperymentalnych, nie badało wpływu ćwiczeń fizycznych, lub nie omawiało wyłącznego efektu suplementacji owocem granatowca. Ostatecznie, do analizy zostało zakwalifikowanych 11 artykułów. Dodatkowe wyszukiwanie w bazie czasopism PubMed nie doprowadziło do identyfikacji żadnych dodatkowych artykułów spełniających kryteria wyboru. Obie autorki przeglądu niezależnie analizowały wybrane artykuły.

WŁAŚCIWOŚCI OWOCU GRANATOWCA

Granatowiec właściwy (*Punica granatum* L.) uprawiany jest na obszarze Azji zachodniej, Kaukazie oraz półwyspie Indyjskim, jednak rozprzestrzenia się również poza tymi regionami. Jego owoc znany jest z wielu korzystnych właściwości. W opublikowanych badaniach przeprowadzonych *in vitro* została udowodniona m.in., jego aktywność przeciwutleniająca i zdolność do przeciwdziałania peroksydacji lipidów (-65%) (Kelawala and Ananthanarayan, 2004). Ponadto wykazano korzystne działanie związków zawartych w soku z granatu na ograniczenie negatywnych skutków promieniowania UV-B (Afaq, Malik, *et al.*, 2005), a także wykazano, że aktywność elagitanin zawartych w jego składzie zapobiega nowotworom piersi

reagującym na estrogeny (Afaq, Malik, *et al.*, 2005). Korzystne właściwości owocu granatowca właściwego zostały również potwierdzone w badaniach *in vivo*. Afaq i Saleem (2005) wykazali zdolność do zapobiegania nowotworom skóry podczas aplikacji naskórnej u myszy modelu CD-1 (Afaq, Saleem, *et al.*, 2005), natomiast w innych badaniach zaobserwowano zahamowanie rozwoju zmian miażdżycowych (Aviram *et al.*, 2002). W badaniach eksperymentalnych prowadzonych na modelu zwierzęcym, wprowadzenie ekstraktu z kwiatu granatowca (500 mg/kg) przez 6 tygodni, spowodowało ograniczenie zwłóknienia serca u myszy Zuckera (Huang *et al.*, 2005). Korzystne efekty suplementacji owocem granatu odnotowane zostały także w innych badaniach klinicznych. Efekty te obejmowały poprawę niedokrwienia mięśnia sercowego wywołanego stresem u pacjentów z chorobą niedokrwienną serca (Sumner *et al.*, 2005) oraz zmniejszenie grubości błony wewnętrznej i środkowej tętnicy szyjnej. Ponadto wykazano redukcję skurczowego ciśnienia krwi u pacjentów ze zwężeniem tętnicy szyjnej (Aviram *et al.*, 2004).

W skład soku z owocu granatowca wchodzą między innymi: antocyjany (Du, Wang i Francis, 1975), flawonoidy (Gómez-Caravaca *et al.*, 2013), kwasy fenolowe, np. kwasy elagowy, galusowy i kofeinowy (Amakura *et al.*, 2000), galusan katechiny i epigallokatechiny (de Pascual-Teresa, Santos-Buelga i Rivas-Gonzalo, 2000), aminokwasy (Lansky i Newman, 2007) i błonnik pokarmowy (nasiona) (Sumaiya, Jahurul i Zzaman, 2018). Jednak jego właściwości przypisuje się głównie wysokiej zawartości polifenoli (~ 3,8 mg / ml) (Seeram *et al.*, 2008), szczególnie kwasu elagowego i elagitanin (80–90%) oraz antocyjanów (8–15%) (Seeram, Lee i Heber, 2004). Dieta bogata w elagitaniny i antocyjany jest związana z redukcją skurczowego ciśnienia krwi oraz wzrostem średnicy naczyń krwionośnych, co w konsekwencji przekłada się na poprawę przepływu krwi przez naczynia krwionośne (Roelofs *et al.*, 2017)(Labonté *et al.*, 2013). Można przypuszczać, że istnieje ścisły związek pomiędzy dietą bogatą w owoce granatu a sprawnością układu krążenia

(Crum *et al.*, 2017). Suplementacja produktami bogatymi w azotany (NO_3^-) wpływa korzystnie na dostarczanie tlenu do komórek podczas wysiłku (Crum *et al.*, 2017). W organizmie NO_3^- podlega konwersji do silnego środka rozszerzającego naczynia, tlenku azotu (II) (NO) (Crum *et al.*, 2017). W stanie spoczynku NO wytwarzany jest głównie endogennie przez syntazy NO, jednak w warunkach niedotlenienia (które mogą występować lokalnie w mięśniach podczas wysiłku) aktywność tego szlaku jest ograniczona, co sprawia, że szlak wtórny, tj. NO_3^- - azotyn (NO_2^-) - NO, nabiera większego znaczenia (Lundberg *et al.*, 1994)(Peri *et al.*, 2005). Ponadto suplementacja polifenolami nasila działanie NO_3^- w diecie, nie tylko poprzez promowanie jego konwersji do NO (Gago *et al.*, 2007)(Ignarro *et al.*, 2006), ale także poprzez ochronę NO przed uszkodzeniami wywołanymi przez działanie wolnych rodników (Lundberg *et al.*, 1994)(Trexler *et al.*, 2014).

Badania przeprowadzone w ostatnich latach wykazały, że suplementy oparte na owocu granatowca mogą wywierać znaczący wpływ na poprawę wykonywania ćwiczeń aerobowych poprzez usprawnienie dostarczania tlenu do włókien mięśniowych u których obserwuje się wzrost zapotrzebowania na ten składnik (Roelofs *et al.*, 2017)(Trexler *et al.*, 2014).

Dodatkową zaletą suplementacji owocami bogatymi w polifenole jest ich zdolność do zwiększania potencjału antyoksydacyjnego, a w konsekwencji zwiększenie całkowitej zdolności antyoksydacyjnej osocza, co ma wpływ na ograniczenie wywołanego intensywnym wysiłkiem fizycznym stanu zapalnego (Urbaniak, *et al.*, 2018).

Dokładny mechanizm biologicznego oddziaływania polifenoli nie jest do tej pory w pełni poznany, chociaż ich korzystny wpływ został niewątpliwie udowodniony, szczególnie w grupach osób narażonych na stres (Afaq, Saleem, *et al.*, 2005)(Aviram *et al.*, 2004)(Shukla *et al.*, 2008)(Polagruto *et al.*, 2004). Do tej grupy z pewnością należałoby zaliczyć osoby uprawiające

sport wyczynowo, ponieważ wysiłek fizyczny stanowi jedną z najbardziej stresujących sytuacji, charakteryzujących się zarówno ostrymi, jak i opóźnionymi zmianami w reakcjach organizmu (Ammar *et al.*, 2018). Zatem, aby zminimalizować niekorzystne, powysiłkowe zmiany indukowane intensywnym i/lub długotrwałym wysiłkiem fizycznym rozważa się wprowadzenie do diety zawodników suplementy, które mogą zminimalizować i/lub przeciwdziałać tym niekorzystnym zjawiskom. Korzystne rezultaty badań, wykazane po suplementacji granatem potwierdzają zasadność wprowadzenia tego owocu również u zawodników. Poniżej przedstawione zostały wyniki wybranych artykułów badawczych poświęconych wpływowi suplementacji owocem granatu u sportowców i osób aktywnych fizycznie.

WPŁYW SUPLEMENTACJI GRANATEM NA WYDOLNOŚĆ FIZYCZNĄ, REAKCJE ODDECHOWE I TERMOREGULACYJNE, OCENĘ POSTRZEGANEGO ZMĘCZENIA I OPÓŹNIONĄ BOLESNOŚĆ MIĘŚNI

Liczne zespoły naukowców prowadziły badania wpływu suplementacji sokiem z owocu granatu na sprawność fizyczną.

W badaniach przeprowadzonych przez zespół profesora Edwarda F. Coyle z University of Texas w Austin grupę 16 rekreacyjnie ćwiczących mężczyzn w wieku 24.2 ± 1.4 lat poddano suplementacji 500 ml soku z granatu dwa razy dziennie, co 12 godzin przez dziewięć dni (Trombold *et al.*, 2010). Jako placebo zastosowano napój zawierający 4 g węglowodanów, przypominający pod względem organoleptycznym sok z granatu (Trombold *et al.*, 2010). W badaniach:

- Nie zaobserwowano statystycznie istotnych różnic w wykonanej pracy ekscentrycznej pomiędzy grupą suplementowaną sokiem z granatu, a otrzymującą placebo.
- W obu grupach (suplementowanej i kontrolnej) odnotowano znaczącą redukcję siły mięśniowej po upływie dwóch godzin od zakończenia wysiłku, która wyniosła odpowiednio 71.5% ± 7.3% i 72.8% ± 10.0%.
- Po 48 godzinach siła uległa znacznej poprawie w obu grupach, jednakże znacznie szybszą regeneracją (pomiędzy 24 a 48 godzinami) odnotowano w grupie suplementowanej sokiem z granatu.
- Wykazano mniejszy spadek siły mięśniowej w grupie otrzymującej sok z granatu (14.6% ± 10.4%), w porównaniu z grupą kontrolną (21.7% ± 10.1%).
- Zaobserwowano statystycznie istotne zmniejszenie odczucia bólu mięśni po upływie dwóch godzin od wysiłku w grupie suplementowanej, w porównaniu z tą otrzymującą placebo.

W innym badaniu przeprowadzonym również przez zespół profesora Edwarda F. Coyle, zastosowano suplementację sokiem z granatu w ilości 250ml, którą spożywano dwa razy dziennie co 12 godzin przez okres 15 dni. Grupę badawczą stanowiło 17 mężczyzn trenujących sporty siłowe, w wieku 21.9 ± 2.4 lat (Trombold *et al.*, 2011). Sok placebo zastosowany w badaniu składał się z 35 g węglowodanów (maltodekstryny i sukralozy) i pod względem organoleptycznym przypominał suplement diety (Trombold *et al.*, 2011). W badaniach tych odnotowano:

- Znaczne zwiększenie siły izometrycznego zginania łokcia w czasie restytucji wynoszącej od 2 do 168 godzin po wysiłku ekscentrycznym (93.6 % w grupie suplementowanej, w porównaniu z 88.9% w grupie otrzymującej placebo).

- Redukcję siły izometrycznej w obu grupach po upływie dwóch godzin od wysiłku.
- Zmniejszenie bólu mięśni zginaczy łokciowych w czasie od dwóch do 168 godzin po wysiłku ekscentrycznym w grupie suplementowanej w porównaniu z grupą otrzymującą placebo.
- Istotną zależność pomiędzy okresem suplementacji sokiem z granatu, a bólem mięśniowym zginacza łokcia.
- Brak istotnych różnic pomiędzy grupą suplementowaną i kontrolną w poziomie siły izometrycznej i odczuwalnej bolesności mięśni w prostownikach kolana.

Następne analizowane badania przeprowadzone zostały pod kierunkiem profesora Edwarda F. Coyle na grupie 12 wytrzymałościowo trenujących mężczyzn w wieku 26.8 ± 5.0 lat (Trinity *et al.*, 2014). Sportowcy otrzymywali 500 ml soku przypominającego składem sok z granatu dziennie co 12 godzin przez siedem dni (Trinity *et al.*, 2014). Podobnie jak w powyższych badaniach jako placebo zastosowano sok przypominający kolorem i smakiem suplement diety, zawierający 4g węglowodanów (maltodekstryny i sukralozy) (Trinity *et al.*, 2014). W badaniach:

- W pierwszym dniu badania nie zaobserwowano znaczących różnic w masie ciała obu testowanych grup sportowców. Pomiar został przeprowadzony dwukrotnie: tuż przed rozpoczęciem ćwiczeń i bezpośrednio po jednej godzinie wysiłku.
- Nie odnotowano także znaczących różnic we współczynnikach wymiany gazowej, wydolności oraz mocy wyjściowej podczas 10 minutowej próby, której zostali obciążeni kolarze w obu grupach.
- Nie zaobserwowano również istotnej różnicy pomiędzy grupą suplementowaną a otrzymującą placebo w zakresie średniej mocy wyjściowej podczas 10 minutowej

próby czasowej (placebo: 292 ± 33 W, grupa suplementowana: 279 ± 38 W), oceną postrzeganego wysiłku po 30 i 45 minutach oraz czasie zmęczenia.

- Pomiar mocy maksymalnej, chwilowej i prędkości przy mocy maksymalnej pozostały niezmienione pomiędzy pierwszym a drugim dniem suplementacji.
- Nie zaobserwowano również różnic pomiędzy grupami w temperaturze ciała i szacowanego tempa przepływu krwi podczas jednogodzinnego treningu.

Profesor Hailee L. Wingfield z University of North Carolina at Chapel Hill wraz ze swoim zespołem przeprowadzili badania na grupie dziewięciu intensywnie ćwiczących kobiet i 10 mężczyzn w wieku 22.0 ± 2.2 lat (Trexler *et al.,* 2014). Jako suplement spożywano ekstrakt z owocu granatowca w dawce 2 x 500 mg, który badani spożyli na 30 min przed wysiłkiem fizycznym (Trexler *et al.,* 2014). Placebo stanowiły kapsułki zawierające 95% maltodekstryny, 5% fioletowej marchwi i dodatek hibiskusa w celach kolorystycznych (Trexler *et al.,* 2014). W badaniach:

- Odnotowano wydłużenie czasu do zmęczenia podczas wykonania wysiłku przy 90% (387.9 ± 199.2 vs. 346.0 ± 162.5 sekundy) i 100% prędkości szczytowej (170.8 ± 66.3 vs. 159.3 ± 62.3).
- Nie zanotowano różnic w odczuciu bólu mięśniowego (mierzonego po wysiłku) pomiędzy grupami.
- Wykazano, że subiektywne odczucie witalności u badanych było wyższe po spożyciu ekstraktu z granatu w porównaniu z placebo.
- Odnotowano znaczące zwiększenie średnicy naczyń krwionośnych 30 min po spożyciu suplementu diety w porównaniu z grupą kontrolną (0.42 ± 0.07 cm vs. 0.39 ± 0.07 cm).

- Zaobserwowano, po spożyciu ekstraktu z granatu, większy przepływ krwi (40.6 ± 24.8 ml / min) w porównaniu z grupą otrzymującą placebo (29.6 ± 24.9 ml / min).

W badaniach Dr Achraf Ammar i współpracowników, które zostały przeprowadzone na zawodnikach reprezentacji olimpijskiej w podnoszeniu ciężarów, zastosowano suplementację sokiem z owocu granatu (Ammar *et al.*, 2016). Zawodnicy przez dwa dni otrzymywali suplement w ilości 750 ml dziennie (dzienna dawka podzielona była na 3 porcje po 250 ml). Z kolei jedną godzinę przed sesją treningową badani spożyli 500 ml soku. Jako placebo zastosowano komercyjnie dostępny napój o smaku granatu, składający się z wody, kwasu cytrynowego, naturalnych substancji smakowych, substancji słodzących (aspartam -0.3 g/l, acesulfamu K -0.16 g/l i stabilizatorów - guma arabska), ale nie zawierającego przeciwutleniaczy, witamin i polifenoli (Ammar *et al.*, 2016). W badaniach:

- Wykazano, że suplementacja poprawiła wydajność ciężarowców olimpijskich o 8.29 ± 3.8% - całkowity podniesiony ciężar i 3.26 ± 0.83% - maksymalny podniesiony ciężar.
- Odnotowano korzystny wpływ suplementacji sokiem z granatu na ocenę postrzeganego wysiłku (-4.37 ± 1.45%) i opóźnionej bolesności mięśni prostowników kolana (- 13.4 ± 3.84%) po upływie 48 godzin od sesji treningowej.

W badaniach opublikowanych przez zespół profesor Meredith G. Mock (University of North Carolina at Chapel Hill) u ośmiu mężczyzn i 11 kobiet, którzy rekreacyjne uprawiali ćwiczenia wysiłkowe (trening siłowy i sprint), zastosowano suplementację ekstraktem z owocu granatu w ilości 1000 mg. Preparat został spożyty przez badanych 30 min przed testem wysiłkowym (Roelofs *et al.*, 2017). Jako placebo wykorzystano kapsułki zawierające 95%

maltodekstryny, 5% fioletowej marchwi i hibiskusa w celach kolorystycznych (Roelofs *et al.*, 2017). W badaniach stwierdzono:

- Pozytywny wpływ suplementacji na średnią moc w próbie szybkości, przy czym analizując poszczególne sesje powtórzeń, największą średnią moc (z dziesięciu sesji) wykazano w sesji piątej.
- Wzrost średnicy naczyń krwionośnych po 30 min od spożycia suplementu diety.
- Pomimo, iż nie odnotowano statystycznie znaczących różnic pomiędzy grupami w ilości powtórzeń wykonanych w wyciskaniu sztangi leżąc oraz wyciskaniu nogami na suwnicy, w obu ćwiczeniach maksymalne ciężary były większe w grupie suplementowanej w porównaniu z grupą otrzymującą placebo.
- Odnotowano wzrost średnicy naczyń krwionośnych w grupie otrzymującej ekstrakt z granatu: bezpośrednio po wyciskaniu sztangi w pozycji leżącej (0.029 cm), po wyciskaniu nogami na suwnicy (0.042 cm), a także po 30 min od zakończenia wysiłku (0.027 cm).

WPŁYW SUPLEMENTACJI GRANATEM NA WYBRANE PARAMETRY BIOCHEMICZNE I REAKCJE SERCOWO-NACZYNIOWE

W przytoczonych powyżej badaniach przeprowadzonych przez zespół profesora Edwarda F. Coyle (Trinity *et al.*, 2014):

- Nie zaobserwowano statystycznie znaczących różnic w stężeniu kwasu mlekowego mierzonego po 5 i 30 minutach od rozpoczęcia treningu oraz po wysiłku.

- W obu badanych grupach, tętno, objętość wyrzutowa serca oraz pojemność minutowa serca w pierwszym i drugim dniu badania podczas 50 minut ćwiczeń i 10 minut po wysiłku kształtowały się na tym samym poziomie.
- Pomimo wzrostu, w porównaniu do wartości spoczynkowych, średnich wartości ciśnienia tętniczego mierzonego podczas końcowych minut każdego submaksymalnego etapu treningu (po 30 i 45 minucie) oraz ponownie w dwu minutowych odstępach, 10 minut po zakończeniu wysiłku, nie odnotowano różnic pomiędzy grupą suplementowaną a kontrolną.

W omawianych przez Ammar i wsp. (2016) badaniach przeprowadzonych na grupie reprezentantów olimpijskich w podnoszeniu ciężarów wykazano, że:

- W grupie suplementowanej pomiędzy wynikami uzyskanymi przed i po sesji treningowej, nastąpił wzrost temperatury ciała (+ 0.42%), obniżenie tętna (- 4.46%), skurczowego ciśnienia krwi (- 1.81%), poziomu glukozy we krwi (- 10.59 ± 3.51%) oraz wzrost poziomu kreatyniny (+ 6.32 ± 1.57%) (Ammar *et al.*, 2016).

W innych badaniach przeprowadzonych przez Crum i wsp. (2017) z Uniwersytetu Massey na dobrze wytrenowanych kolarzach (siedmiu mężczyzn i jedna kobieta), zastosowano suplementację ekstraktem z owocu granatu w dawce 1000 mg, która została spożyta przed treningiem. Jako placebo zastosowano kapsułki tego samego koloru, wielkości i kształtu co suplement, zawierające brązowy cukier (Crum *et al.*, 2017). Analiza otrzymanych wyników wskazała na:

- Wzrost stężenia NO_3^- w osoczu (+ 10,3 μmol) w grupie suplementowanej ekstraktem z granatu w porównaniu z grupą otrzymującą placebo.

- Brak wpływu suplementacji na wartość skurczowego ciśnienia krwi oraz poziomu VO_2, VCO_2, tętna i stężenia kwasu mlekowego.

W badaniach przeprowadzonych przez grupę badawczą profesor Vicente-Salar (2016) zastosowanie suplementacji sokiem z granatu (w dawce 200 ml dziennie przez 21 dni, przy czym jedna grupa otrzymała sam sok w tej dawce, natomiast druga grupa otrzymała 100 ml soku + 100 ml wody) przyczyniło się do:

- Istotnie statystycznego wzrostu poziomu glukozy mierzonej pomiędzy 0 a 22 dniem badań w grupie kontrolnej i w grupie suplementowanej (Fuster-Muñoz *et al.*, 2016).
- W tym samym przedziale czasowym statystycznie istotnego wzrostu poziomu mleczanu i spadku poziomu ferrytyny w grupie suplementowanej (Fuster-Muñoz *et al.*, 2016).
- Statystycznie zwiększonego poziomu cholesterolu wysokiej gęstości (HDL-C) na koniec eksperymentu w grupie suplementowanej sokiem z owocu granatu : H_2O 1:1 (Fuster-Muñoz *et al.*, 2016).
- Znaczącego wzrostu stężania jonów K^+ pod koniec badań jedynie w grupie suplementowanej (Fuster-Muñoz *et al.*, 2016).

WPŁYW SUPLEMENTACJI GRANATEM NA PARAMETRY USZKODZENIA WŁÓKIEN MIĘŚNIOWYCH, STRESU OKSYDACYJNEGO I MARKERY STANU ZAPALNEGO

W kolejnych zaprezentowanych w pracy badaniach, opublikowanych przez Mazani i wsp. (2014), zastosowano u intensywnie ćwiczących mężczyzn (18-24 lat) sok z granatu w ilości 240 ml (lub takiej samej ilości wody kranowej w grupie placebo) (Mazani *et al.,* 2014). Omawiani autorzy odnotowali:

- Znaczny spadek poziomu markerów stanu zapalnego w surowicy, w tym: białka C-reaktywnego, metaloproteinazy macierzy 2 i 9 w grupie suplementowanej.
- Znaczny wzrost poziomów peroksydazy glutationowej (GPX) i dysmutazy ponadtlenkowej we krwi oraz poziomu całkowitej zdolności antyoksydacyjnej w surowicy w grupie suplementowanej po interwencji.
- Spadek zawartości dialdehydu malonowego (MDA) w surowicy (biomarker peroksydacji lipidów) w grupie suplementowanej w porównaniu z grupą kontrolną.

W omawianej już wcześniej pracy Ammar i wsp. (2016), która analizowała wpływ podaży soku z granatu u zawodników wysokokwalifikowanych zaobserwowano również:

- Znaczny wzrost poziomu kinazy kreatynowej (+ 19.53%) i dehydrogenazy mleczanowej (LDH) (+ 14.61%) mierzony po treningu w odniesieniu do wartości uzyskanych przed treningiem (Ammar *et al.*, 2016).
- Brak wpływu suplementacji na przed – po treningowy poziom aminotransferazy asparaginianowej, a także fosforanu alkalicznego oraz białka C-reaktywnego.

- Wzrost następujących markerów w grupie otrzymującej placebo: aminotransferaza asparaginianowa (+ 16,59%), fosforan alkaliczny (+ 04,51%) i białko C-reaktywne (+ 12,59%).
- Znaczący, opóźniony wpływ suplementacji sokiem z granatu na wartości kinazy kreatynowej i LDH przed treningiem, gdzie zaobserwowano niższe wartości dla grupy suplementowanej w porównaniu z grupą otrzymującą placebo.
- W okresie restytucji, mierzonej po 3 minutach i 48 godzinach po zakończeniu sesji treningowej, wykazano korzystny wpływ na poziom kinazy kreatynowej (11.34 ± 1.98%), LDH (7.30 ± 0.86%) i aminotransferazy asparaginianowej (6.77 ± 0.47%).

W opisanych powyżej badaniach opublikowanych przez grupę badawczą profesor Vicente-Salar (2016) zaobserwowano:

- Znaczący spadek poziomu aminotransferazy asparaginianowej (z 29.5 do 23.5 U/l) oraz wzrost stężenia białkowych grup karbonylowych (1.1 do 1.8 nmol/mg) i MDA (z 10.9 do 14.1 nmol/g białka) w grupie kontrolnej między dniem 0 a 22 (Fuster-Muñoz *et al.*, 2016).
- Obniżenie poziomu MDA po 21 dniach suplementacji 200 ml soku z granatu dziennie oraz 200 ml soku z granatu w proporcjach 1:1 z H_2O w dawce dziennej.

W innym badaniu przeprowadzonym również przez Dr Achraf Ammar na grupie dziewięciu ciężarowców olimpijskich zaobserwowano:

- Podwyższony poziom MDA przed – po sesji treningowej w obu grupach, jednakże w grupie suplementowanej wzrost ten był mniejszy w porównaniu z grupą otrzymującą placebo (Ammar *et al.*, 2017).

- Wzrost antyoksydacyjnych biomarkerów enzymatycznych: katalazy (CAT) i GPX oraz nieenzymatycznych: kwasu moczowego (UA) i bilirubiny (Tbil), w obu grupach przed – po sesji treningowej, przy czym wzrostem w grupie otrzymującej sok z granatu był większy.
- Istotną zależność pomiędzy sesją treningową a suplementacją i stężeniami MDA, CAT i UA.
- Poziom markerów peroksydacji lipidów oraz markerów antyoksydacyjnych (CAT, GPX, UA i Tbil) uległ obniżeniu w obu grupach pomiędzy 3 minutami a 48 godzinami po zakończeniu sesji treningowej - z wyższymi wskaźnikami spadku po suplementacji sokiem z granatu w porównaniu do placebo (współczynniki spadku Δ = 5.63%, 8.94%, 10.21%, 3.57% i 7.42% odpowiednio dla MDA, CAT, GPX, UA i Tbil).
- 48-godzinny okres pełnej regeneracji, jako wystarczający czas w połączeniu z suplementacją granatem, do przywrócenia wszystkich parametrów do wartości uzyskanych przed wysiłkiem.

PUBLIKACJA 2

METODOLOGIA

W badaniach własnych przeprowadzonych na Młodzieżowej Reprezentacji Polski w Wioślarstwie zawodnicy z grupy suplementowanej spożywali codziennie przez 6 tygodni po 50 ml soku z granatu. Grupa kontrolna otrzymywała w tej samej dawce i czasie placebo (50 ml płynu dziennie składającego się z wody, cukru i grenadyny przypominający organoleptycznie zastosowany suplement diety).

WPŁYW SUPLEMENTACJI SOKIEM Z OWOCU GRANATOWCA WŁAŚCIWEGO NA ZDOLNOŚĆ ANTYOKSYDACYJNĄ OSOCZA I GOSPODARKĘ ŻELAZEM U SPORTOWCÓW TRENUJĄCYCH WYCZYNOWO WIOŚLARSTWO

Trening fizyczny, zwłaszcza w sporcie wyczynowym charakteryzuje się znacznym obciążeniem organizmu zawodnika i przyczynia się do przyspieszonego starzenia się erytrocytów. Towarzyszące wysiłkowi fizycznemu takie procesy jak: hypertermia, kwasica metaboliczna, hipoglikemia i hemokoncentracja, dodatkowo zmniejszają oporność osmotyczną erytrocytów (Chatard *et al.,* 1999) (Reeder i Wilson, 2001) (Seeram and Nair, 2002). Yusof i wsp., [2007] sugerują, że hemoliza obserwowana w trakcie długotrwałych wysiłków jest spowodowana uszkodzeniem starszych erytrocytów, które są mniej elastyczne, a tym samym bardziej podatne na uszkodzenia (Yusof *et al.,* 2007). Cytowani autorzy wykazali również bardzo wysoką ujemną

korelację (r = - 0.911, p <0.05) pomiędzy poziomem spektryny (białko peryferyjne błony erytrocytu, występujące na wewnętrznej powierzchni tej błony, tworzące tzw. szkielet błonowy) a hemolizą – potwierdzając tezę, że strukturalne zmiany w błonie, będące wynikiem zwiększenia generacji wolnych rodników, zwiększają wrażliwość erytrocytów na rozpad (Yusof *et al.*, 2007). Intensywne, długotrwałe wysiłki fizyczne mogą być przyczyną wzmożonej hemolizy wysiłkowej, w rezultacie w osoczu obserwuje się wzrost poziomu wolnego żelaza - biokatalizatora reakcji wolnorodnikowych (Martínez *et al.*, 2006) (Martínez *et al.*, 2006) i zapalnych (Peeling *et al.*, 2009).

Wzrost stężenia zjonizowanego żelaza w surowicy krwi, będący następstwem tego procesu, przyczynić się może zarówno do nasilenia reakcji wolnorodnikowych z jednej strony (Bresgen i Eckl, 2018), a z drugiej wpływać na osłabienie układu odpornościowego zwiększając tym samym podatność na infekcję (BALTOPOULOS, 2009) (Walsh i Oliver, 2016); (Ward *et al.*, 2011). Opisana w literaturze powysiłkowa depresja układu odpornościowego skutkować może nie tylko zwiększoną częstością infekcji u zawodników, ale również wyższym odsetkiem zachorowań (szczególnie na schorzenia górnych dróg oddechowych - URTI) i znacznie dłuższym czasem ich trwania (Gleeson i Pyne, 2016). Zatem zastosowanie polifenoli, które wykazują zdolność chelatowania jonów żelaza, może wpływać nie tylko na redukcję stresu oksydacyjnego, ale również ograniczyć immunosupresję spowodowaną intensywnym wysiłkiem fizycznym. Procesy wolnorodnikowe mogą pełnić pośrednią rolę w regulacji procesów zapalnych indukowanych intensywnymi ćwiczeniami fizycznymi, natomiast kluczową rolę w przebiegu tychże procesów odgrywać może metabolizm żelaza. Interesujące dane uzyskano w pracach nad hepcydyną – hormonem biorącym udział w regulacji homeostazy żelaza w organizmie. Wykazano, że stan zapalny i związane z nim cytokiny (przede wszystkim IL-6) stymulują produkcję hepcydyny, która

wpływa na obniżanie poziomu żelaza w organizmie stając się przyczyną niedokrwistości (Nemeth, Rivera, *et al.*, 2004).

Zastosowana w badaniach wioślarzy suplementacja sokiem z granatu zwiększyła potencjał antyoksydacyjny mierzony poziomem TAC. W grupie suplementowanej, w okresie restytucji, poziom tego parametru był statystycznie istotnie wyższy niż w grupie kontrolnej (Fig.1A). Jednakże wzmocnienie potencjału antyoksydacyjnego nie miało istotnego wpływu na pozostałe analizowane parametry. Z badań Gil i wsp. (Gil *et al.*, 2000) wynika, że sok z granatu charakteryzuje się trzykrotnie wyższą aktywnością antyoksydacyjną w porównaniu do innych, powszechnie uznanych produktów spożywczych o działaniu antyoksydacyjnym, takich jak czerwone wino czy zielona herbata. Wysoki potencjał antyoksydacyjny soku z granatu jest wynikiem obecności związków polifenolowych, a w szczególności proantocyanin (El Kar *et al.*, 2011).

Intensywny wysiłek fizyczny spowodował u badanych zawodników, w I terminie badań (przed suplementacją), istotną redukcję poziomu całkowitych antyoksydantów (Fig. 1). Nadmierna koncentracja, niedostatecznie unieczynnionych wolnych rodników, może m.in., inicjować peroksydację wielonienasyconych kwasów tłuszczowych znajdujących się w błonach erytrocytów, a tym samym powodować wzrost hemolizy po wysiłku (Beneke *et al.*, 2005) (Bonilla, Narváez i Chuaire, 2005). Wykazany u badanych wioślarzy, przed suplementacją, wyższy powysiłkowy wzrost poziomu żelaza (Fig. 2c) może potwierdzać powyższą tezą. Manthou i wsp. (Manthou *et al.*, 2017), w badaniach przeprowadzonych u zdrowych osób wykazali, że 14 dniowa suplementacja sokiem z granatu przyczyniła się do wzrostu ilości krwinek czerwonawych (RBC), zawartości hemoglobiny i hematokrytu. Zdaniem autorów te korzystne zmiany wynikają z ochrony krwinek czerwonych przed ich degradacją. Fiorani i wsp. (Fiorani, Accorsi i Cantoni, 2003)

wykazali, że ludzkie erytrocyty, na zasadzie biernej dyfuzji, mogą pobierać ze środowiska flawonoidy stanowiąc swoisty rezerwuar tych związków. Największy odsetek flawonoidów przedostaje się do cytozolu (autorzy szacują, że aż 85% początkowej ilości flawonoidów), a część zostaje wbudowana w błonę komórkową. Z badań (Arora *et al.*, 2000)(Terao, Piskula i Yao, 1994) wynika, że flawonoidy podobnie jak cholesterol i α- tokoferol lokalizują się w pobliżu warstwy błonowej pomiędzy dwuwarstwą lipidową i fazą wodną. Takie usytuowanie flawonoidów odgrywa bardzo ważną rolę, ponieważ wpływa na stabilizację błon biologicznych, które na skutek ograniczenia ich płynności stają się bardziej odporne na działanie czynników utleniających (Cherubini, Beal i Frei, 1999). Bardzo istotna jest również kooperacja pomiędzy flawonoidami a α-tokoferolem i kwasem askorbinowym. Wykazano bowiem, że flawonoidy wpływają na zahamowanie utleniania α-tokoferolu w komórce oraz regenerują (podobnie jak witamina C) utleniony α-tokoferol do formy rodnikowej. Z kolei kwas askorbinowy, którego utlenieniu także przeciwdziałają flawonoidy, może wpływać na zahamowanie oksydatywnych przemian flawonoidów przedłużając tym samym ich ochronne działanie (Block, Henson i Levine, 1991)(Clemetson i Andetsen, 1966). Zatem utrzymanie przez flawonoidy względnej równowagi pomiędzy utlenionymi i zredukowanymi postaciami antyoksydantów wraz z ich formami rodnikowymi stanowi kolejny czynnik zabezpieczający organizm przed zwiększonym stężeniem rodników tlenowych pochodnych innego antyoksydantu.

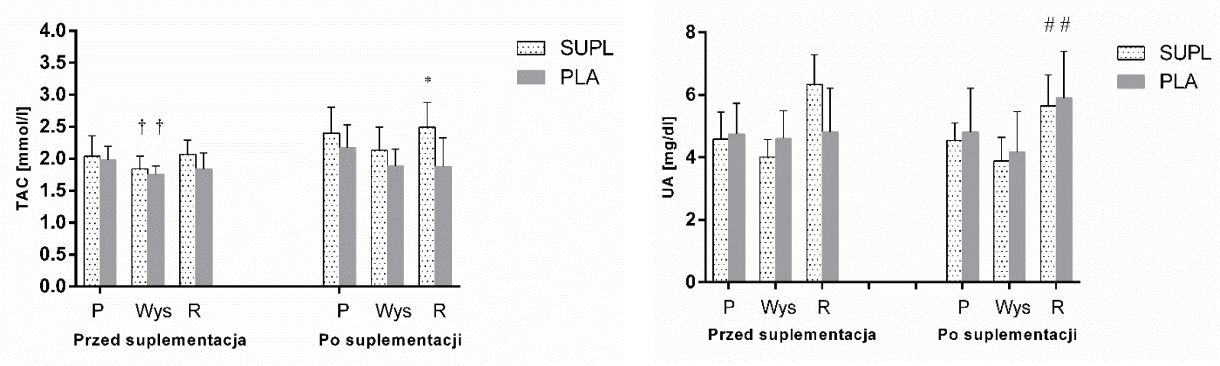
Pomimo wzmocnienia potencjału antyoksydacyjnego w grupie suplementowanej, w II terminie badań, zastosowany u zawodników intensywny test ergometryczny nie wpłynął na poziom TAC w żadnej z analizowanych grup (Fig. 1a). Również kwas moczowy jako końcowy produkt metabolizmu puryn wskazywany w badaniach *in vivo* jako ważny antyoksydant osocza, stanowiąc jednocześnie jego największą składową (Kaur i Halliwell, 1990) nie przyczynił się do

zmian poziomu TAC pomimo, że w badaniach wioślarzy stwierdzono wzrost tego parametru w okresie restytucji (Fig. 1b).

Analizując uzyskane w badaniach własnych wyniki można domniemać, że istotnym czynnikiem, który może mieć również wpływ na poziom TAC jest okres treningowy. II termin badań przypadał bowiem na okres startowy w którym organizm dobrze wytrenowanych zawodników powinien cechować się tzw. „gotowością startową", czyli pełną adaptacją do tego rodzaju wysiłku fizycznego. Adaptację zawodników do większych obciążeń wysiłkowych potwierdzają również pozostałe analizowane parametry, a mianowicie brak statystycznie istotnych zmian w poziomie IL-6 (Fig. 4) czy też powysiłkowego wzrostu poziomu żelaza (Fig. 2c) w analizowanym okresie. Main i wsp. (Main *et al.*, 2010) przeprowadzając badania na mężczyznach, członkach kadry olimpijskiej w wioślarstwie, zauważyli istotny związek pomiędzy poziomem cytokin prozapalnych IL-1β, TNF-α oraz IL-6 a obniżeniem nastroju, zaburzeniami snu i odczuciem zmęczenia. A zatem brak istotnych statystycznie zmian w poziomie cytokin prozapalnych, pod wpływem wysiłku fizycznego, może stanowić ważną informację dotyczącą przygotowania zawodników do zawodów.

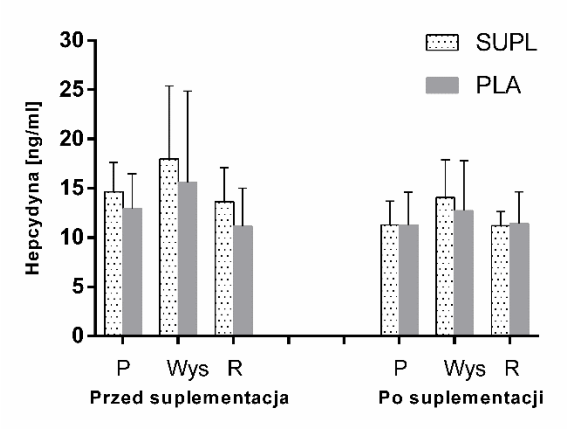
W przeprowadzonych badaniach nie zaobserwowano statystycznie znamiennych różnic w parametrach gospodarki żelazem pomiędzy grupą badaną, a suplementowaną w II terminie badań, co również potwierdzać może powyższą tezę. Zatem zastosowanie u zawodników, którzy podlegają intensywnym obciążeniom wysiłkowym, suplementacji preparatami bogatymi w antocyjany może stanowić dodatkowe zabezpieczenie mające korzystny wpływ na dynamiczny układ immunologiczny.

a. **b.**

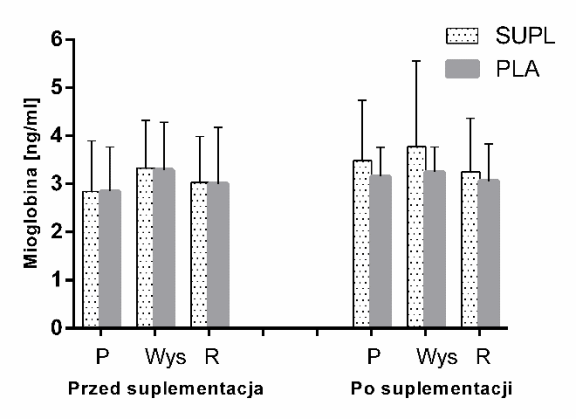


Rysunek 1 Dane przedstawiają średnie wartości całkowitej zdolności antyoksydacyjnej (TAC) (**a**) i stężenia kwasu moczowego (UA) (**b**) podczas przeprowadzonych testów wysiłkowych przed i po suplementacji (średnia ± odchylenie standardowe średniej). SUPL - grupa suplementowana; PLA – grupa otrzymująca placebo; P - pomiar przed wysiłkiem; Wys – pomiar bezpośrednio po wysiłku; R - pomiar po 24 godzinnym czasie regeneracji; † - statystycznie istotna różnica w porównaniu z pomiarem wyjściowym ; * - statystycznie istotna różnica w porównaniu z PLA; # - statystycznie istotna różnica w porównaniu z poziomem po wysiłku

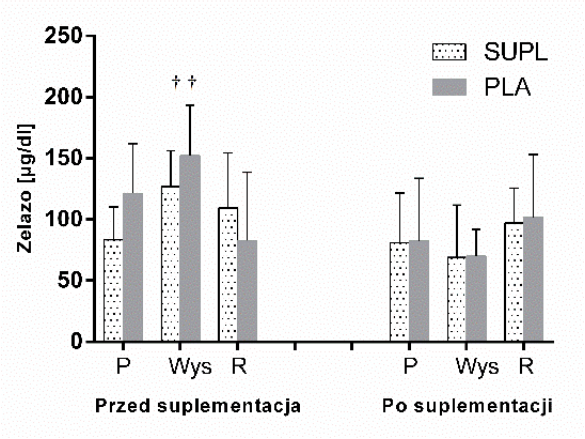
a.



b.

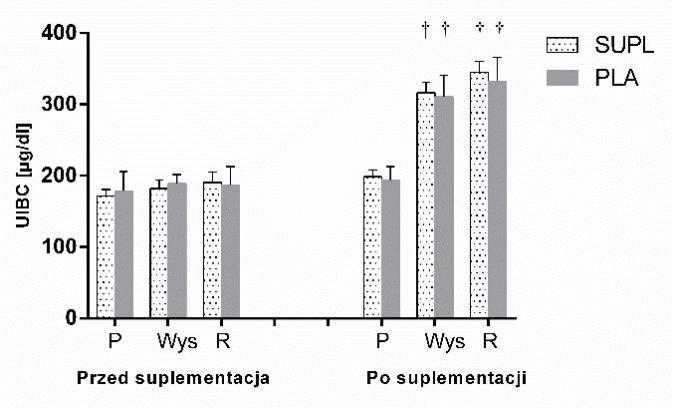


c.

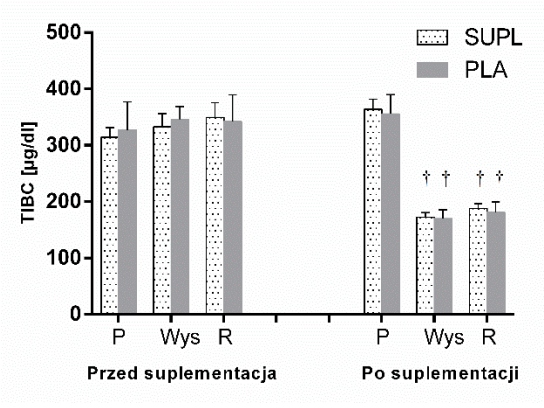


Rysunek 2 Dane przedstawiają średnie wartości stężenia hepcydyny (**a**), mioglobiny (**b**), i żelaza (**c**) podczas przeprowadzonych testów wysiłkowych przed i po suplementacji (średnia ± odchylenie standardowe średniej). SUPL - grupa suplementowana; PLA – grupa otrzymująca placebo; P - pomiar przed wysiłkiem; Wys – pomiar bezpośrednio po wysiłku; R - pomiar po 24 godzinnym czasie regeneracji; † - statystycznie istotna różnica w porównaniu z pomiarem wyjściowym

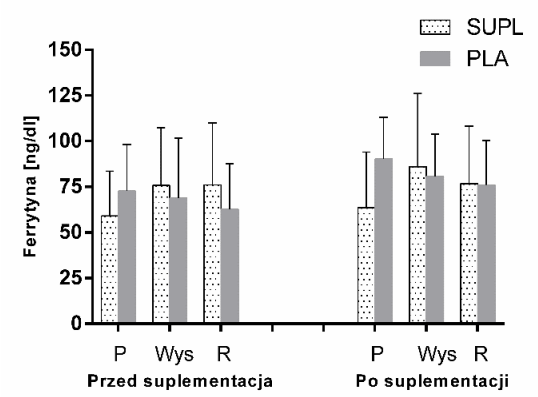
a.



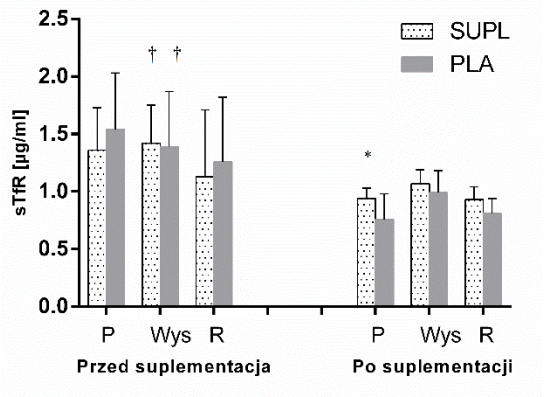
b.



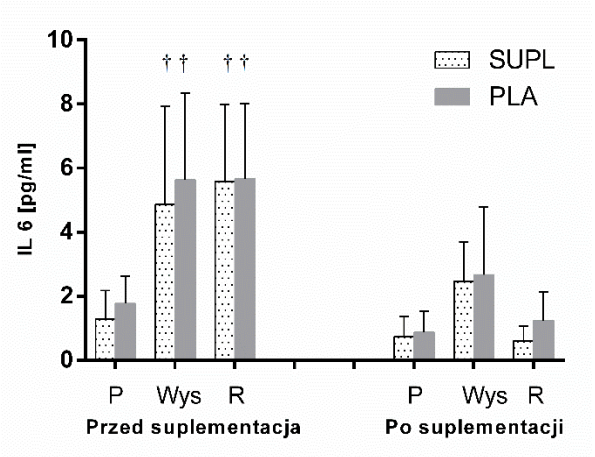
c.



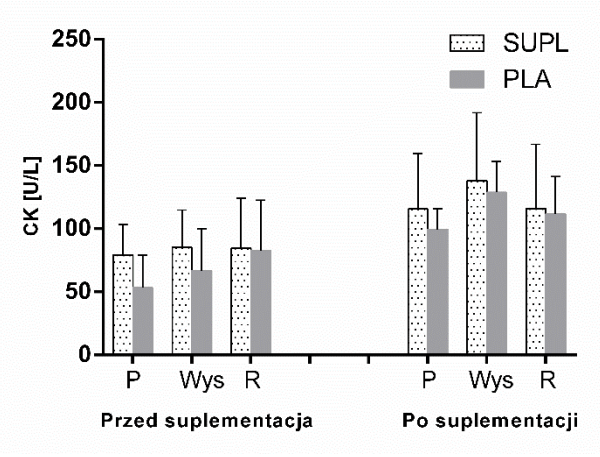
d.



Rysunek 3 Dane przedstawiają średnie wartości stężenia utajonej zdolności wiązania żelaza (UIBC) (**a**), całkowitej zdolności wiązania żelaza (TIBC) (**b**), ferrytyny (**c**) i rozpuszczalnych receptorów transferyny (sTfR) (**d**) podczas przeprowadzonych testów wysiłkowych przed i po suplementacji (średnia ± odchylenie standardowe średniej). SUPL - grupa suplementowana; PLA – grupa otrzymująca placebo; P - pomiar przed wysiłkiem; Wys – pomiar bezpośrednio po wysiłku; R - pomiar po 24 godzinnym czasie regeneracji; † - statystycznie istotna różnica w porównaniu z pomiarem wyjściowym; * - statystycznie istotna różnica w porównaniu z PLA



Rysunek 4 Dane przedstawiają średnie wartości stężenia interleukiny 6 (IL-6) podczas przeprowadzonych testów wysiłkowych przed i po suplementacji (średnia ± odchylenie standardowe średniej). SUPL - grupa suplementowana; PLA – grupa otrzymująca placebo; P - pomiar przed wysiłkiem; Wys – pomiar bezpośrednio po wysiłku; R - pomiar po 24 godzinnym czasie regeneracji; † - statystycznie istotna różnica w porównaniu z pomiarem wyjściowym



Rysunek 5 Dane przedstawiają średnie wartości stężenia kinazy kreatynowej (CK) podczas przeprowadzonych testów wysiłkowych przed i po suplementacji (średnia ± odchylenie standardowe średniej). SUPL - grupa suplementowana; PLA – grupa otrzymująca placebo; P - pomiar przed wysiłkiem; Wys – pomiar bezpośrednio po wysiłku; R - pomiar po 24 godzinnym czasie regeneracji

WNIOSKI

Uzyskane wyniki przedstawiłam w formie dwóch oryginalnych publikacji naukowych. Na podstawie literatury w publikacji przeglądowej pt. "*Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic revie*w" opisałam dotychczasowe wyniki dotyczące suplementacji owocem granatu wśród sportowców i osób aktywnych fizycznie. Natomiast w publikacji pt. "*The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers*" przedstawiłam własne wyniki badań dotyczące wpływu suplementacji sokiem z owocu granatu na wybrane parametry wolnorodnikowe i gospodarkę żelazem u sportowców trenujących wyczynowo wioślarstwo.

W wyniku przeprowadzonych analiz można stwierdzić, że:

- W większości badań dedykowanych wpływowi suplementacji owocem granatu wśród sportowców i osób aktywnych fizycznie udowodniono jego korzystne właściwości między innymi na: poprawę siły całego ciała, odczuwalne uczucie witalności, redukcję zmęczenia i bólu mięśni, wzrost średnicy naczyń krwionośnych, obniżenie tempa wzrostu tętna, skurczowego ciśnienia krwi, stężenia kinazy kreatynowej oraz zwiększenie aktywności enzymów przeciwutleniających (peroksydazy glutationowej i dysmutazy ponadtlenkowej),
- Suplementacja sokiem z owocu granatowca miała korzystny wpływ na podniesienie poziomu TAC u sportowców trenujących wyczynowo wioślarstwo,
- Suplementacja sokiem z owocu granatowca nie miała znaczącego wpływu na markery stanu zapalnego u wioślarzy.

Otrzymane przeze mnie wyniki poszerzyły obecny stan wiedzy na temat suplementacji owocem granatu wśród sportowców i dowodzą, o jego korzystnym wpływie na podniesienie poziomu całkowitej pojemności antyoksydacyjnej u sportowców trenujących wyczynowo wioślarstwo. Jednakże poznanie dokładnych mechanizmów działania związków zawartych w owocach granatowca na organizm wymagać będzie przeprowadzenia dalszych, szczegółowych badań.

Lista skrótów

CAT – katalaza

CK – kinaza kreatynowa

GPX – peroksydaza glutationowa

HDL-C – cholesterol wysokiej gęstości

IL-6 – interleukina 6

LA – kwas mlekowy

LDH – dehydrogenaza mleczanowa

MDA – dialdehyd malonowy

PLA – grupa otrzymująca placebo

sTfR – rozpuszczalne receptory transferyny

TIBC – całkowita zdolność wiązania żelaza

TAC – całkowita pojemność antyoksydacyjna

Tbil – bilirubina

UA – kwas moczowy

UIBC – utajona zdolność wiązania żelaza

II. DISSERTATION SUMMARY

The basis of this doctoral dissertation is a series of publications under the collective title: "Effect of supplementation with pomegranate fruit juice (*Punica granatum* L.) on antioxidant capacity of plasma and iron metabolism in rowers", which includes two scientific articles published in international journals.

1. **A. Urbaniak**, A. Skarpańska-Stejnborn, Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic review, ***Int. J. Vitam. Nutr. Res.*** 1-15 (**2019**) – 5 year impact factor – 1.251, ministry score – 100 pkt.

2. **A. Urbaniak**, P. Basta, K. Ast, A. Wołoszyn, J. Kuriańska-Wołoszyn, E. Latour, A. Skarpańska-Stejnborn, The impact of supplementation with pomegranate fruit (*Punica granatum* L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers, ***J. Int. Soc. Sport. Nutr.*** 15:35 (**2018**) – 5 year impact factor – 3.508, ministry score – 100 pkt.

INTRODUCTION

The aim of the study was to analyze the impact of pomegranate juice (*Punica granatum* L.) supplementation as a potentially effective, achievable and safe therapy to reduce the harmful effects of intense physical exercise on high-performance athletes.

When intense physical exercise presents a significant load on an athlete's body, it can lead to disturbances in internal homeostasis as well as negatively affect competitive results. One of the

key factors that affects an athlete's performance is iron metabolism (Buratti *et al.*, 2015) (Otto, Montgomery and Richards, 2013). Iron is involved in many physiological processes, such as oxygen transport and energy acquisition (Gozzelino and Arosio, 2016). Disturbances in iron homeostasis may be initiated not only by its deficiency in the diet, but also by the inflammation caused by intense physical exercise (Dahlquist *et al.*, 2017). Inflammation in the body is associated with increased synthesis of hepcidin – a hormone involved in the degradation of ferroportin, which minimizes the migration of iron from cellular reserves (in liver and spleen) and reduces iron absorption from the gastrointestinal tract (Nemeth, Tuttle, *et al.*, 2004) (Sukumaran *et al.*, 2012). It should be emphasized that the persistence of such condition may result in reduced erythropoiesis and, as a consequence, lead to the development of anemia (Liu *et al.*, 2011). The current research shows that, at the end of the sports season, up to 70% of athletes (rowers and soccer players) have functional serum iron deficiency, and 27% have an absolute serum deficiency of this element (Reinke *et al.*, 2012).

Supplementation with iron-rich dietary products is not always beneficial, and its results may be harmful (Clénin *et al.*, 2016) (Ishibashi *et al.*, 2017). In *in vivo* studies, intramuscular iron injection resulted in an increase of the concentration of this element in muscles and plasma and an increase of oxidative stress. Consequently, these animals were characterized by significantly lower muscle strength than the control group (Reardon and Allen, 2009).

An increase in post-exercise level of hepcidin may also be associated with an increase of unbounded iron pool released from damaged erythrocytes (Buratti *et al.*, 2015) (Berzosa *et al.*, 2011). It should be emphasized that this unbounded iron can catalyze free radical reactions leading to further damage of erythrocytes.

Recent studies have found a beneficial effect of pomegranate juice supplementation on iron deficiency related disorders in humans (Shema-Didi *et al.*, 2013). These results contributed to the growing interest in pomegranate fruit supplementation in athletes (Ammar *et al.*, 2018).

Pomegranate fruit juice is a rich source of polyphenols. These include primarily: anthocyanins, flavonols and some elagitanins. From those, anthocyanins are characterized by a number of biological activities, including: antioxidant (CĂta *et al.*, 2016), stimulating the immune system, anti-inflammatory (Karlsen *et al.*, 2007), as well as iron chelating, thus limiting the pool of unbounded ions (Kelsey *et al.*, 2011).

AIM OF THE STUDIES PRESENTED IN TWO ARTICLES

The aim of the study presented in the review article "Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic review" was a comparison of previous results related with pomegranate fruit supplementation in athletes and physically active subjects.

The aim of the studies presented in the research article "The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers" was to understand the mechanism of iron metabolism during and after intense physical exercise and the role of flavonoids contained in pomegranate juice in this process. In particular, the research aimed to answer the question: whether pomegranate juice supplementation rich in polyphenols will reduce inflammation and whether and to what extent these changes will affect markers of iron metabolism in athletes.

The hypothesis of this doctoral dissertation was based on the assumption that pomegranate juice supplementation, by increasing the antioxidant potential, may contribute to the reduction of inflammation induced by intense physical exercise in highly trained rowers. It was also analyzed whether and to what extent these changes affected selected parameters of iron metabolism.

PUBLICATION 1

METHODOLOGY

The present review article was written according to the guidelines published by Pautasso (Pautasso, 2013). The results were analyzed in the agreement with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyzes) guidelines. The articles included in the review were identified through the Web of Science database using the following search terms in the title: "pomegranate supplementation", "pomegranate exercise", "pomegranate sport" or "Punicagranatum L. supplementation". The publications cited in the selected articles were also taken into consideration. In order to ensure the best quality, articles published before 2000, written in a language other than English, conference abstracts, communications, and other review publications were excluded. Selected articles included only clinical studies related to the impact of pomegranate fruit supplementation in athletes or physically active subjects. Unpublished research and scientific dissertations were not included in the review. Initially, 58 articles were identified based on a database search, and an additional four based on cited references. 53 publications were selected after the duplicates were removed. 18 of them were excluded based on the type of article (communication, conference abstract, etc.), 17 did not concern clinical trials, six

did not examine the impact of physical exercise, and five did not discuss the exclusive effect of pomegranate supplementation. Eleven articles were finally qualified. Additional searches in the PubMed journal database did not identify any additional publications that meet the selection criteria. Both authors of the review independently analyzed selected articles.

POMEGRANATE FRUIT

Pomegranate (*Punica granatum* L.) is grown in Western Asia, the Caucasus and the Indian Peninsula, but it also spreads outside these regions. Its fruit is known for many beneficial properties. Its antioxidant activity, the ability to counteract lipid peroxidation (-65%)(Kelawala and Ananthanarayan, 2004) and to reduce the negative effects of UV-B radiation (Afaq, Malik, *et al.*, 2005), was shown *in vitro*. Moreover, the activity of elagitanins contained in pomegranate fruit was shown to prevent estrogen-responsive breast cancer (Afaq, Malik, *et al.*, 2005). In *in vivo* studies, Afaq and Saleem (2005) demonstrated epidermal application of pomegranate fruit to prevent skin tumors in CD-1 mice (Afaq, Saleem, *et al.*, 2005), while other studies reported inhibition of atherosclerotic lesions in mice (Aviram *et al.*, 2002), as well as reduction of cardiac fibrosis in Zucker mice after six weeks of treatment with pomegranate flower extract (500 mg / kg) (Huang *et al.*, 2005). Beneficial effects of pomegranate supplementation have also been reported in clinical trials. Those included improved stress-induced myocardial ischemia in patients with ischemic heart disease (Sumner *et al.*, 2005) and a reduction of carotid intima-media thickness and systolic blood pressure in patients with carotid artery stenosis (Aviram *et al.*, 2004).

Pomegranate fruit juice contains anthocyanins (DU, WANG and FRANCIS, 1975), flavonoids(Gómez-Caravaca *et al.*, 2013), phenolic acids, e.g. ellagic, gallic and caffeic acids

(Amakura *et al.*, 2000), catechin and epigallocatechin gallate (de Pascual-Teresa, Santos-Buelga and Rivas-Gonzalo, 2000), amino acids (Lansky and Newman, 2007) and dietary fiber (seeds) (Sumaiya, Jahurul and Zzaman, 2018). However, its beneficial properties are mainly linked to the high content of polyphenols (~ 3.8 mg / ml) (Seeram *et al.*, 2008), especially ellagic acid and elagitanins (80-90%) and anthocyanins (8-15%) (Seeram, Lee and Heber, 2004). It was previously shown that elagitanins and anthocyanins rich diet is associated with a reduction in systolic blood pressure and an increase in the diameter of blood vessels, which results in the improved blood flow through the blood vessels (Roelofs *et al.*, 2017) (Labonté *et al.*, 2013). It can be assumed that there is a close relationship between a diet rich in pomegranate fruit and cardiovascular health (Crum *et al.*, 2017). Supplementation with nitrate-rich (NO_3^-) dietary products has a positive effect on the oxygen supply to cells during physical exercise (Crum *et al.*, 2017). NO_3^- is being converted to a strong vasodilator, nitric oxide (NO) (Crum *et al.*, 2017). At rest, NO is mainly produced endogenously by NO synthases, however, under hypoxia, which may occur locally in the muscles during exercise, the activity of this pathway is limited, which makes the secondary pathway, i.e. NO_3^- - nitrite (NO_2^-) - NO, more significant (Lundberg *et al.*, 1994) (Peri *et al.*, 2005). Additionally, polyphenol supplementation enhances the effects of NO_3^- in the diet, not only by promoting its conversion to NO (Gago *et al.*, 2007) (Ignarro *et al.*, 2006), but also by protecting NO from the damage induced by free radical species (Lundberg *et al.*, 1994) (Trexler *et al.*, 2014).

Recent studies have shown that pomegranate fruit supplementation has significant impact in improving aerobic exercise performance by boosting oxygen delivery to muscles (Roelofs *et al.*, 2017)(Trexler *et al.*, 2014).

An additional advantage of polyphenol rich fruits supplementation is its ability to increase the antioxidant potential and, consequently, increase the total antioxidant capacity of plasma which

consequently minimizes the inflammation induced by intense physical exercise (Urbaniak, Basta, Ast, Wołoszyn, Kuriańska – Wołoszyn, *et al.*, 2018).

The exact mechanism of the biological activity of polyphenols is not fully understood yet, however, their beneficial effect has been shown, especially in subjects exposed to stressful conditions (Afaq, Saleem, *et al.*, 2005) (Aviram *et al.*, 2004) (Shukla *et al.*, 2008) (Polagruto *et al.*, 2004). These results prompted many researchers to conduct studies on the relationship between pomegranate fruit supplementation and physical exercise since it is one of the most stressful conditions, characterized by both acute and delayed changes in body reactions (Ammar *et al.*, 2018). Therefore, in order to minimize adverse, post-exercise changes induced by intense and / or prolonged physical exercise, it is recommended to include dietary supplements in the athlete's diet that can minimize and / or counteract these adverse effects. Positive results of the studies on pomegranate fruit supplementation justify its inclusion in athlete's diet. Below, I am presenting the summary of the findings from selected research articles dedicated to the impact of pomegranate fruit supplementation on athletes and physically active participants.

EFFECT OF POMEGRANATE FRUIT SUPPLEMENTATION ON PHYSICAL PERFORMANCE, RESPIRATORY AND THERMOREGULATORY RESPONSES, THE RATING OF PERCEIVED EXERTION AND DELAYED ONSET SORENESS

Numerous studies have been dedicated to the impact of supplementation with pomegranate fruit on physical fitness.

In a study conducted by the research group of Dr. Edward F. Coyle from the University of Texas in Austin, 16 recreationally active men aged 24.2 ± 1.4 years were supplemented with 500 ml of pomegranate juice twice a day, with12 hour intervals for nine days (Trombold *et al.*, 2010). A drink containing 4 g of carbohydrates, which resembles in color and taste pomegranate juice was employed as a placebo (Trombold *et al.*, 2010). It was shown that:

- There were no statistically significant differences in the eccentric exercise between supplemented and placebo groups.
- In both groups there was a significant reduction in muscle strength two hours after exercise, 71.5% ± 7.3% and 72.8% ± 10.0%, respectively.
- After 48 hours, strength improved significantly in both groups, however, much faster recovery (between 24 and 48 h) was observed in the supplemented group. This resulted in the higher strength in the supplemented group vs the placebo group, 85.4% ± 10.1% vs 78.3% ± 10.1% after 48 h and 88.9% ± 7.9% vs 84.0% ± 7.8% after 72 h.

- The decrease in strength from baseline to 48 hours after exercise was significantly lower in the supplemented group (14.6% ± 10.4%) vs placebo (21.7% ± 10.1%) with concomitant improved recovery as compared to the control group.
- A statistically significant reduction in muscle soreness was observed 2 h after exercise in the supplemented group as compared to placebo group.

In another study conducted as well by the team of Professor Edward F. Coyle, a group of 17 resistance trained men aged 21.9 ± 2.4 years was supplemented with 250 ml of pomegranate juice twice a day every 12 hours for a period of 15 days (Trombold *et al.*, 2011). The placebo used in the study consisted of 35 g carbohydrates (maltodextrin and sucralose) and resembled a dietary supplement (Trombold *et al.*, 2011). The main conclusions of the work are as follow:

- There was a significant increase in the isometric elbow flexion strength during 2 - 168 hours period after eccentric exercise (93.6% in the supplemented group vs 88.9% in the placebo group).
- A significant reduction in isometric strength was observed in both groups two hours after exercise.
- Pomegranate juice supplementation significantly reduced perceptible elbow flexor muscle pain within two to 168 hours after eccentric exercise compared to the placebo group.
- A significant relationship was observed between the pomegranate juice supplementation and the reduction of post-eccentric exercise elbow flexor soreness. Measurements at 48 and 72 hours after exercise for supplemented vs placebo group were as follow: 2.71 ± 2.11 vs. 3.77 ± 1.68 and 1.65 ± 2.09 vs. 2.41 ± 1.70.

- No significant difference in isometric strength and muscle soreness in knee extensors was observed in both groups.

The next analyzed research published by Professor Edward F. Coyle research group were conducted on 12 endurance trained men aged 26.8 ± 5.0 years (Trinity *et al.*, 2014). Athletes received 500 ml of juice resembling the composition of pomegranate daily every 12 hours for seven days (Trinity *et al.*, 2014). Similar to the above studies, juice containing 4g of carbohydrates (maltodextrin and sucralose) resembling a dietary supplement in color and taste was used as a placebo (Trinity *et al.*, 2014). The main findings from this research include as follow:

- On the first day of the study, no significant differences in body weights of both groups of athletes were observed. The measurement was carried out twice: just before starting the exercises and after one hour of exercise.
- There were also no significant differences in respiratory exchange ratio and gross efficiency during cycling performance for both groups.
- No significant differences were observed between the trials in average power output during the 10 minute time trial (placebo: 292 ± 33 and supplemented group: 279 ± 38 W), ratings of perceived exertion at 30 and 45 minutes, and the time of fatigue.
- Measurement of maximum power, instantaneous power and velocity at maximal power remained unchanged between the first and second day of supplementation.
- There were also no pair-wise differences between groups at core and skin temperature or estimated blood flow rate during one hour of exercise.

The team of Professor Hailee L. Wingfield from the University of North Carolina at Chapel Hill conducted a study on a group of highly active participants, nine women and ten men, aged 22.0 ± 2.2 years (Trexler *et al.*, 2014). As a supplement, pomegranate extract 2 x 500 mg was

consumed 30 minutes before exercise (Trexler *et al.*, 2014). As a placebo, capsules containing 95% maltodextrin, 5% purple carrot and the addition of hibiscus as colorant was used (Trexler *et al.*, 2014). The main observation from this research include:

- There was a beneficial effect of consumption of two portions of 500 mg of pomegranate extract 30 minutes before exercise to increase time to exhaustion at 90% (387.9 ± 199.2 vs. 346.0 ± 162.5 seconds) and 100% peak velocity (170.8 ± 66.3 vs. 159.3 ± 62.3).
- There were no significant differences in the level of perceived pain between the groups.
- On the vitality scale, as demonstrated by the following statement: "At this moment I feel alive and vital," it was found that vitality is significantly higher 30 minutes after ingesting pomegranate extract compared to placebo.
- A significant increase in blood vessel diameter was recorded 30 minutes after ingestion of the dietary supplement compared to the control group (0.42 ± 0.07 cm vs. 0.39 ± 0.07 cm).
- A significant increase in blood flow was observed 30 minutes after supplementation with pomegranate extract (40.6 ± 24.8 ml / min) compared to the placebo group (29.6 ± 24.9 ml / min).

In the research conducted by Ammar et al., Olympic weightlifters received pomegranate juice in a dose of 750 ml daily for two days (the daily dose was divided into 3 portions of 250 ml) (Ammar *et al.*, 2016). Additionally, one hour before the training session, the subjects consumed 500 ml of juice extra (Ammar *et al.*, 2016). As a placebo, a commercially available pomegranate drink was used, consisting of water, citric acid, natural flavors, sweeteners (aspartame (0.3 g / l),

acesulfame K (0.16 g / l), stabilizers (acacia), but not containing antioxidants, vitamins and polyphenols (Ammar *et al.*, 2016). The main findings from the studies include:

- Supplementation with pomegranate juice was shown to improve the performance of Olympic weightlifters by 8.29 ± 3.8% - total weight and 3.26 ± 0.83% - maximum weight.
- Beneficial effects of pomegranate juice supplementation on ratings of perceived exertion (-4.37 ± 1.45%) and delayed onset of muscle soreness of knee extensors (-13.4 ± 3.84%) were noted 48 hours after the training session.

In a study published by the group of Professor Meredith G. Mock from the University of North Carolina at Chapel Hill, recreationally resistant trained participants, eight men and 11 women, were supplemented with 1000 mg of pomegranate extract 30 minutes before the sprint test (Roelofs *et al.*, 2017). Capsules containing 95% maltodextrin, 5% purple carrot and hibiscus as colorant were used as placebo (Roelofs *et al.*, 2017). The main findings of the research include:

- Pomegranate extract supplementation has positive effect on the average power in a sprint test, with significantly higher power on the fifth of ten sprints.
- There was a significant increase in blood vessel diameter 30 min after ingestion of the dietary supplement.
- It was observed that ingestion of pomegranate extract resulted in increased initial and 30 min post-exercise blood flow (compared to 30 min post-ingestion) and increased blood flow immediately after exercise compared to baseline.
- Although there were no statistically significant differences in the number of repetitions performed in bench press and leg press between groups, in both

exercises the maximum weights were higher in the supplemented group compared to the placebo.

- A significantly larger diameter of the blood vessels was observed in the group supplemented with pomegranate extract immediately after bench press (0.029 cm), immediately after leg press (0.042 cm) and 30 min after exercise (0.027 cm).

EFFECT OF POMEGRANATE FRUIT SUPPLEMENTATION ON BIOLOGICAL PARAMETERS AND CARDIOVASCULAR RESPONSES

In the studies conducted by the group of Professor Edward F. Coyle described above (Trinity *et al.*, 2014):

- There were no statistically significant differences in the concentration of lactic acid after 5 and 30 minutes from the exercise.
- In both study groups, heart rate, stroke volume and cardiac output responses were at the same level on the first and second day of the study during the 50 minutes of exercise and 10 minutes after exercise.
- There was a significant increase in mean arterial pressure during the exercise compared to resting phase, but no difference was seen between the groups.

In the study conducted on Olympic weightlifters by (Ammar *et al.*, 2016) it was shown that:

- In the supplemented group there was a significant acute effect on the increase in body temperature (+ 0.42%), a decrease in heart rate (- 4.46%), systolic blood

pressure (- 1.81%), blood glucose (- 10.59 ± 3.51%) and an increase in creatinine levels (+ 6.32 ± 1.57%) in a pre-post training session (Ammar *et al.*, 2016).

In another study conducted by Crum et al. (2017) from the University of Massey, eight highly trained cyclists (seven men and one woman) were supplemented with 1000 mg of pomegranate extract before workout (Crum *et al.*, 2017). Capsules of the same color, size and shape containing brown sugar were used as a placebo (Crum *et al.*, 2017). The following observations were made:

- Increase in plasma NO_3^- concentration (+ 10.3 μmol) in the group supplemented with pomegranate extract compared to the placebo group.
- No effect of supplementation on systolic blood pressure, VO_2, VCO_2, heart rate and lactic acid levels.

In studies conducted by the research group of Professor Vicente-Salar (2016) on endurance-based athletes, the supplementation with pomegranate juice (200 ml per day for 21 days, with one group receiving juice alone, while the other group received 100 ml of juice + 100 ml of water) contributed to:

- Statistically significant increase in glucose level measured between 0 and 22 days in both groups (Fuster-Muñoz *et al.*, 2016).
- In the same time interval statistically significant increase in lactate level and decrease in ferritin level in the supplemented group (Fuster-Muñoz *et al.*, 2016).
- Statistically increased high-density lipoprotein cholesterol level (HDL-C) at the end of the experiment in the group supplemented with pomegranate juice: H_2O 1:1 (Fuster-Muñoz *et al.*, 2016).

- A significant increase in K^+ ion concentration at the end of the study only in the supplemented group (Fuster-Muñoz *et al.*, 2016).

EFFECT OF POMEGRANATE FRUIT SUPPLEMENTATION ON MUSCLE DAMAGE PARAMETERS, OXIDATIVE STRESS AND INFLAMMATORY MARKERS

In studies published by Mazani et al. (2014) 28 exhaustive exercised subjects aged 18-24 years were supplemented with 240 ml of pomegranate juice (or the same amount of tap water in the placebo group) (Mazani *et al.*, 2014). The main observations from the study include:

- A significant decrease in the level of inflammatory markers in the serum, including: C-reactive protein, matrix metalloproteinases 2 and 9 in the supplemented group.
- Significant increase in blood glutathione peroxidase (GPX) and superoxide dismutase levels as well as total serum antioxidant capacity in the supplemented group after intervention.
- Significant decrease in serum malondialdehyde (MDA) level (lipid peroxidation biomarker) in the supplemented group compared to the control group.

In the previously discussed work of Ammar et al. (2016), which analyzed the impact of pomegranate juice supplementation in highly qualified Olympic weightlifters, they also observed:

- Significant acute increase in creatine kinase (+ 19.53%) and lactate dehydrogenase (LDH) (+ 14.61%) pre-post training session (Ammar *et al.*, 2016).
- Lack of acute effect of supplementation on pre- and post-workout levels of aspartate aminotransferase, alkaline phosphate, and C-reactive protein.

- Increase in the following markers in the placebo group: aspartate transaminase (+ 16.59%), alkaline phosphate (+ 04.51%) and C-reactive protein (+ 12.59%) in pre-post training session.
- Significant delayed effect of pomegranate juice supplementation on pre-workout creatine kinase and LDH values, where lower values were observed for the supplemented group compared to the placebo group.
- Positive effect of supplementation on improving regeneration between 3 minutes and 48 hours after a training session for creatine kinase (11.34 ± 1.98%), LDH (7.30 ± 0.86%) and aspartate aminotransferase (6.77 ± 0.47%).

In the previously described studies published by the research group Professor Vicente-Salar (2016) it was observed:

- Significant decrease in aspartate aminotransferase (from 29.5 to 23.5 U / l) and an increase in the concentration of protein carbonyl groups (1.1 to 1.8 nmol / mg) and MDA (from 10.9 to 14.1 nmol / g protein) in the control group between days 0 and 22 (Fuster-Muñoz *et al.*, 2016).
- Decrease in the level of MDA after 21 days of supplementation with 200 ml pomegranate juice daily and 200 ml pomegranate juice: H_2O 1:1 daily.

From another study also conducted by Dr. Achraf Ammar on a group of nine Olympic weightlifters the following observations were made:

- Increased pre-post MDA levels in both groups with a smaller increase in the supplemented group compared to the placebo group (Ammar *et al.*, 2017).

- Pre-post training session increase in enzymatic: catalase (CAT) and GPX and non-enzymatic: uric acid (UA) and bilirubin (Tbil) antioxidants, in both groups, with a larger increase in the group receiving pomegranate juice.
- Significant interaction between training session and supplementation on MDA, CAT and UA concentrations.
- Reduction of lipid peroxidation markers, and antioxidant markers (CAT, GPX, UA and Tbil) in both groups between 3 minutes and 48 hours after the training session, with higher decrease rates after pomegranate juice supplementation compared to placebo (decrease rates Δ = 5.63 %, 8.94%, 10.21%, 3.57% and 7.42% for MDA, CAT, GPX, UA and Tbil respectively).
- 48-hour recovery period was reported as sufficient to restore all parameters to resting values in the pomegranate juice supplemented group.

PUBLICATION 2

METHODOLOGY

In my research conducted on the group of 19 male rowers, members of the Polish National Team, athletes were supplemented with 50 ml of pomegranate juice daily for six weeks. The control group received placebo (50 ml fluid daily consisting of water, sugar and grenadine, resembling dietary supplement) at the same dose and time.

EFFECT OF SUPPLEMENTATION WITH POMEGRANATE FRUIT JUICE ON ANTIOXIDANT CAPACITY OF PLASMA AND IRON METABOLISM IN ROWERS

Physical exercise, especially competitive, is a significant load on the athlete's body and contributes to enhanced erythrocyte aging. Accompanying to the physical exercise processes such as hyperthermia, metabolic acidosis, hypoglycemia and hemoconcentration further reduce osmotic resistance of erythrocytes (Chatard *et al.*, 1999) (Reeder and Wilson, 2001) (Seeram and Nair, 2002). Yusof et al. [2007] suggested that hemolysis observed during prolonged exercise is caused by the damage induced to older erythrocytes that are less flexible and thus more susceptible to breakage (Yusof *et al.*, 2007). The same authors also showed a very high negative correlation ($r = - 0.911$, $p < 0.05$) between the level of spectrin (peripheral protein of erythrocyte membrane, present on the inner surface of this membrane, forming the so-called membrane skeleton) and hemolysis - confirming the thesis that structural changes in the membrane, resulting from increased free radical generation, increase erythrocyte sensitivity to degradation (Yusof *et al.*, 2007). Intense, prolonged physical exercise can lead to increased exercise induced hemolysis and consequently boost the level of free iron in plasma - a free radical (Martínez *et al.*, 2006) and inflammatory (Peeling *et al.*, 2009) biocatalyst.

The increase in the serum concentration of ionized iron, which is a consequence of this process, can contribute to both intensification of free radical reactions (Bresgen and Eckl, 2018) and affecting immune system therefore increasing susceptibility to infection (BALTOPOULOS, 2009) (Walsh and Oliver, 2016) (Ward *et al.*, 2011). Previously described in the literature, post-exercise depression of the immune system may result not only in the increased frequency of

infections but also higher percentage of such cases (especially in upper respiratory tract diseases – URTI) (Gleeson and Pyne, 2016). For this reason, the consumption of polyphenols exhibiting iron chelating properties can not only reduce oxidative stress but also reduce immunosuppression caused by intense physical exercise. Free radical processes may play an indirect role in the regulation of inflammatory reactions induced by intense physical exercise, while iron metabolism may play a key role in those processes. Interesting data was obtained in the work on hepcidin - a hormone involved in the regulation of iron homeostasis in the body. Inflammation, and associated with it cytokines (primarily IL-6), have been shown to stimulate hepcidin production which reduces iron levels in the body and consequently causes anemia (Nemeth, Rivera, *et al.*, 2004).

Pomegranate juice supplementation increased the antioxidant potential measured by TAC levels in highly trained rowers. In the supplemented group, during the restitution period, the level of this parameter was significantly higher than in the placebo group (Urbaniak *et al.*, 2018, Fig. 1A). However, increase in the antioxidant potential had no significant effect on the other parameters analyzed. The research of Gil et al. (Gil *et al.*, 2000) shows that pomegranate juice is characterized by three times higher antioxidant activity compared to other food products widely recognized for their antioxidant activity, such as red wine or green tea. The high antioxidant potential of pomegranate juice is the consequence of the high content of polyphenolic compounds, in particular proanthocyanins (El Kar *et al.*, 2011).

Intense physical exercise in the first test period (before supplementation) lead to significant reduction of the total antioxidants level in rowers (Urbaniak *et al.*, 2018, Fig. 1A). Excessive concentration of insufficiently inactivated free radicals can lead to initiation of the peroxidation of polyunsaturated fatty acids present in erythrocyte membranes thus causing an increase in hemolysis (Beneke *et al.*, 2005) (Bonilla, Narváez and Chuaire, 2005). Post-exercise increase in

iron levels before supplementation in rowers (Urbaniak *et al.*, 2018, Fig. 2C) may confirm the above thesis. Manthou et al. (Manthou *et al.*, 2017) showed that 14-day supplementation with pomegranate juice contributed to an increase in the number of red blood cells (RBC), hemoglobin and hematocrit in healthy people. According to the authors, this beneficial effect is a consequence of the protection of red blood cells from their degradation. Fiorani et al. (Fiorani, Accorsi and Cantoni, 2003) have shown that human erythrocytes can absorb flavonoids by passive diffusion, from the environment, constituting a specific reservoir of these compounds. The highest percentage of flavonoids gets absorbed into the cytosol (the authors estimate that as much as 85% of the initial amount of flavonoids), and some are being built into the cell membrane. It has been shown that flavonoids, similar to cholesterol and α-tocopherol, are located near the membrane between the lipid bilayer and the aqueous phase (Arora *et al.*, 2000) (Terao, Piskula and Yao, 1994). This location plays a very important role since it affects the stabilization of biological membranes, which due to the limitation of their fluidity become more resistant to oxidizing agents (Cherubini, Beal and Frei, 1999). The other important factor is cooperation between flavonoids and α-tocopherol and ascorbic acid. Flavonoids have been shown to inhibit the oxidation of α-tocopherol in the cell and regenerate (similarly to vitamin C) oxidized α-tocopherol to its radical form. In turn, ascorbic acid, which oxidation is also counteracted by flavonoids, can affect the inhibition of oxidative changes of flavonoids, thereby prolonging their protective effect (Block, Henson and Levine, 1991) (Clemetson and Andetsen, 1966). Thus, the maintenance of a balance by flavonoids between oxidized and reduced forms of antioxidants together with their radical forms is another factor protecting the body against the increased concentration of oxygen radicals derived from another antioxidant.

Despite the strengthening of the antioxidant potential in the supplemented group, in the second term of the study, the intensive ergometric test did not affect the TAC level in any of the analyzed groups (Urbaniak *et al.*, 2018, Fig. 1A). Also, uric acid as the final product of purine metabolism as indicated in *in vivo* studies as an important plasma antioxidant (Kaur and Halliwell, 1990) did not contribute to changes in TAC levels, although an increase in this parameter during the restitution period was observed (Urbaniak *et al.*, 2018, Fig. 1B).

Analyzing the results obtained in my own study, it can be presumed that an important factor that can also affect the level of TAC is the training period. The second test period was held during the competitive period when the body of well-trained athletes is characterized by full adaptation to this type of physical exercise. The rowers' adaptation to higher exercise loads is also confirmed by other analyzed parameters, namely the lack of statistically significant changes in the level of IL-6 (Urbaniak *et al.*, 2018, Fig. 4) or post-exercise iron level increase (Urbaniak *et al.*, 2018, Fig. 2C) during the analyzed period. In the study conducted by Main et al. (Main *et al.*, 2010), a significant relationship between the levels of pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 and depressed mood, sleep disorders and the feeling of fatigue was observed in a team of Olympic rowers. Therefore, the lack of statistically significant changes in the level of pro-inflammatory cytokines, after physical exercise, may constitute important information regarding the preparation of athletes for the competition.

Moreover, in the conducted studies, no statistically significant differences in iron metabolism parameters were observed between the placebo and supplemented group in the second study period, which may also confirm the above thesis. Therefore, the supplementation with anthocyanin-rich products in athletes who are subjected to intense physical exercise may provide additional protection for the dynamic immune system.

CONCLUSIONS

Obtained results have been published as two original scientific articles. Based on the current literature findings in the review entitled "Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic review" I described the results regarding pomegranate supplementation in athletes and physically active subjects. Whereas in the publication entitled "The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers" I presented results from my own research on the impact of pomegranate juice supplementation on selected free radical parameters and iron metabolism in rowers.

Based on the analyzed results, the following conclusions can be presented:

- In most of the studies dedicated to the effect of pomegranate supplementation in athletes and physically active subjects, its beneficial effects have been proven. Those include: improvement of the total body strength and feeling of vitality, reduction of fatigue and muscle pain, increase in blood vessel diameter, decrease in pulse rate, systolic blood pressure and creatine kinase levels and increase in the levels of antioxidant enzymes (glutathione peroxidase and superoxide dismutase),
- Pomegranate juice supplementation had a beneficial effect on increasing the TAC level in competitive rowers,
- Pomegranate juice supplementation had no significant effect on inflammatory markers in competitive rowers.

The obtained results extended the current state of knowledge about pomegranate supplementation in athletes and prove its beneficial effect on increasing the level of total

antioxidant capacity in competitive rowers. However, understanding the exact mechanisms of activity of the compounds contained in pomegranate fruit on the athlete's body will require further more detailed research.

List of abbreviations

CAT – catalase

CK – creatine kinase

GPX – glutathione peroxidase

HDL-C – high density lipoprotein cholesterol

IL-6 – interleukin 6

LA – lactic acid

LDH – lactate dehydrogenase

MDA – malon dialdehyde

PLA – placebo group

sTfR – soluble transferrin receptors

TIBC – total iron binding capacity

TAC – total antioxidant capacity

Tbil – total bilirubin

UA – uric acid

UIBC – unsaturated iron binding capacity

Literatura / Bibliography

Afaq, F., Saleem, M., *et al.* (2005) 'Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-κB pathways and inhibits skin tumorigenesis in CD-1 mice', *International Journal of Cancer*. doi: 10.1002/ijc.20587.

Afaq, F., Malik, A., *et al.* (2005) 'Pomegranate Fruit Extract Modulates UV-B–mediated Phosphorylation of Mitogen-activated Protein Kinases and Activation of Nuclear Factor Kappa B in Normal Human Epidermal Keratinocytes¶', *Photochemistry and Photobiology*. doi: 10.1562/2004-08-06-ra-264.1.

Amakura, Y. *et al.* (2000) 'Determination of phenolic acids in fruit juices by isocratic column liquid chromatography', *Journal of Chromatography A*. doi: 10.1016/S0021-9673(00)00625-7.

Ammar, A. *et al.* (2016) 'Pomegranate supplementation accelerates recovery of muscle damage and soreness and inflammatory markers after a weightlifting training session', *PLoS ONE*. doi: 10.1371/journal.pone.0160305.

Ammar, A. *et al.* (2017) 'Effects of pomegranate juice supplementation on oxidative stress biomarkers following weightlifting exercise', *Nutrients*. doi: 10.3390/nu9080819.

Ammar, A. *et al.* (2018) 'Effects of pomegranate supplementation on exercise performance and post-exercise recovery in healthy adults: a systematic review', *British Journal of Nutrition*. doi: 10.1017/s0007114518002696.

Arora, A. *et al.* (2000) 'Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids', *Archives of Biochemistry and Biophysics*. doi: 10.1006/abbi.1999.1525.

Aviram, M. *et al.* (2002) 'Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans.', *Drugs under experimental and clinical research*.

Aviram, M. *et al.* (2004) ‘Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation’, *Clinical Nutrition*. doi: 10.1016/j.clnu.2003.10.002.

BALTOPOULOS, P. (2009) ‘Exercise induced modulation of immune system functional capacity.’, *Journal Biology of Exercise*. doi: 10.4127/jbe.2009.0024.

Beneke, R. *et al.* (2005) ‘Haemolysis caused by alterations of α and β-spectrin after 10 to 35 min of severe exercise’, *European Journal of Applied Physiology*. doi: 10.1007/s00421-005-0010-y.

Berzosa, C. *et al.* (2011) ‘Erythrocyte membrane fluidity and indices of plasmatic oxidative damage after acute physical exercise in humans’, *European Journal of Applied Physiology*. doi: 10.1007/s00421-010-1738-6.

Block, G., Henson, D. E. and Levine, M. (1991) ‘Vitamin C: A new look’, *Annals of Internal Medicine*. doi: 10.7326/0003-4819-114-10-909.

Bonilla, J. F., Narváez, R. and Chuaire, L. (2005) ‘Sports as a cause of oxidative stress and hemolysis | El deporte como causa de estrés oxidativo y hemólisis’, *Colombia Medica*.

Bresgen, N. and Eckl, P. (2018) ‘Oxidative stress and cell death: The role of iron’, *Free Radical Biology and Medicine*. doi: 10.1016/j.freeradbiomed.2018.05.012.

Buratti, P. *et al.* (2015) ‘Recent advances in iron metabolism: Relevance for health, exercise, and performance’, *Medicine and Science in Sports and Exercise*. doi: 10.1249/MSS.0000000000000593.

CĂta, A. *et al.* (2016) ‘Evaluation of antioxidant activities of some small fruits containing anthocyanins using electrochemical and chemical methods’, *Croatica Chemica Acta*. doi: 10.5562/cca2656.

Chatard, J. C. *et al.* (1999) ‘Anaemia and iron deficiency in athletes. Practical recommendations

for treatment', *Sports Medicine*. doi: 10.2165/00007256-199927040-00003.

Cherubini, A., Beal, M. F. and Frei, B. (1999) 'Black tea increases the resistance of human plasma to lipid peroxidation in vitro, but not ex vivo', *Free Radical Biology and Medicine*. doi: 10.1016/S0891-5849(99)00064-7.

Clemetson, C. A. B. and Andetsen, L. (1966) 'PLANT POLYPHENOLS AS ANTIOXIDANTS FOR ASCORBIC ACID', *Annals of the New York Academy of Sciences*. doi: 10.1111/j.1749-6632.1966.tb19021.x.

Clénin, G. E. *et al.* (2016) 'Iron deficiency in sports - definition, influence on performance and therapy', *Schweizerische Zeitschrift fur Sportmedizin und Sporttraumatologie*.

Crum, E. M. *et al.* (2017) 'The effect of acute pomegranate extract supplementation on oxygen uptake in highly-trained cyclists during high-intensity exercise in a high altitude environment', *Journal of the International Society of Sports Nutrition*. doi: 10.1186/s12970-017-0172-0.

Dahlquist, D. T. *et al.* (2017) 'Effects of macro- And micronutrients on exercise-induced hepcidin response in highly trained endurance athletes', *Applied Physiology, Nutrition and Metabolism*. doi: 10.1139/apnm-2017-0207.

DU, C. T., WANG, P. L. and FRANCIS, F. J. (1975) 'ANTHOCYANINS OF POMEGRANATE, Punica granatum', *Journal of Food Science*. doi: 10.1111/j.1365-2621.1975.tb02217.x.

Fiorani, M., Accorsi, A. and Cantoni, O. (2003) 'Human red blood cells as a natural flavonoid reservoir', *Free Radical Research*. doi: 10.1080/10715760310001615998.

Fuster-Muñoz, E. *et al.* (2016) 'Effects of pomegranate juice in circulating parameters, cytokines, and oxidative stress markers in endurance-based athletes: A randomized controlled trial', *Nutrition*. doi: 10.1016/j.nut.2015.11.002.

Gago, B. *et al.* (2007) 'Red wine-dependent reduction of nitrite to nitric oxide in the stomach', *Free Radical Biology and Medicine*. doi: 10.1016/j.freeradbiomed.2007.06.007.

Gil, M. I. *et al.* (2000) 'Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing', *Journal of Agricultural and Food Chemistry*. doi: 10.1021/jf000404a.

Gleeson, M. and Pyne, D. B. (2016) 'Respiratory inflammation and infections in high-performance athletes', *Immunology and Cell Biology*. doi: 10.1038/icb.2015.100.

Gómez-Caravaca, A. M. *et al.* (2013) 'Determination of the major phenolic compounds in pomegranate juices by HPLC-DAD-ESI-MS', *Journal of Agricultural and Food Chemistry*. doi: 10.1021/jf400684n.

Gozzelino, R. and Arosio, P. (2016) 'Iron homeostasis in health and disease', *International Journal of Molecular Sciences*. doi: 10.3390/ijms17010130.

Huang, T. H. W. *et al.* (2005) 'Pomegranate flower extract diminishes cardiac fibrosis in zucker diabetic fatty rats: Modulation of cardiac endothelin-1 and nuclear factor-kappaB pathways', *Journal of Cardiovascular Pharmacology*. doi: 10.1097/01.fjc.0000190489.85058.7e.

Ignarro, L. J. *et al.* (2006) 'Pomegranate juice protects nitric oxide against oxidative destruction and enhances the biological actions of nitric oxide', *Nitric Oxide - Biology and Chemistry*. doi: 10.1016/j.niox.2006.03.001.

Ishibashi, A. *et al.* (2017) 'Iron supplementation during three consecutive days of endurance training augmented hepcidin levels', *Nutrients*. doi: 10.3390/nu9080820.

El Kar, C. *et al.* (2011) 'Pomegranate (punica granatum) juices: Chemical composition, micronutrient cations, and antioxidant capacity', *Journal of Food Science*. doi: 10.1111/j.1750-3841.2011.02211.x.

Karlsen, A. *et al.* (2007) 'Anthocyanins Inhibit Nuclear Factor-κB Activation in Monocytes and Reduce Plasma Concentrations of Pro-Inflammatory Mediators in Healthy Adults', *The Journal of Nutrition*. doi: 10.1093/jn/137.8.1951.

Kaur, H. and Halliwell, B. (1990) 'Action of biologically-relevant oxidizing species upon uric acid. Identification of uric acid oxidation products', *Chemico-Biological Interactions*. doi: 10.1016/0009-2797(90)90006-9.

Kelawala, N. S. and Ananthanarayan, L. (2004) 'Antioxidant activity of selected foodstuffs', *International Journal of Food Sciences and Nutrition*. doi: 10.1080/09637480400015794.

Kelsey, N. *et al.* (2011) 'Neuroprotective effects of anthocyanins on apoptosis induced by mitochondrial oxidative stress', *Nutritional Neuroscience*. doi: 10.1179/1476830511Y.0000000020.

Labonté, K. *et al.* (2013) 'Acute Effects of Polyphenols from Cranberries and Grape Seeds on Endothelial Function and Performance in Elite Athletes', *Sports*. doi: 10.3390/sports1030055.

Lansky, E. P. and Newman, R. A. (2007) 'Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer', *Journal of Ethnopharmacology*. doi: 10.1016/j.jep.2006.09.006.

Liu, Y. Q. *et al.* (2011) 'Does hepatic hepcidin play an important role in exercise-associated anemia in rats?', *International Journal of Sport Nutrition and Exercise Metabolism*. doi: 10.1123/ijsnem.21.1.19.

Lundberg, J. O. N. *et al.* (1994) 'Intragastric nitric oxide production in humans: Measurements in expelled air', *Gut*. doi: 10.1136/gut.35.11.1543.

Main, L. C. *et al.* (2010) 'Relationship between inflammatory cytokines and self-report measures of training overload', *Research in Sports Medicine*. doi: 10.1080/15438621003627133.

Manthou, E. *et al.* (2017) 'Effect of pomegranate juice consumption on biochemical parameters and complete blood count', *Experimental and Therapeutic Medicine*. doi: 10.3892/etm.2017.4690.

Martínez, A. C. *et al.* (2006) 'Hand strike-induced hemolysis and adaptations in iron metabolism in basque ball players', *Annals of Nutrition and Metabolism*. doi: 10.1159/000090742.

Mazani, M. *et al.* (2014) 'Effect of pomegranate juice supplementation on matrix metalloproteinases 2 and 9 following exhaustive exercise in young healthy males', *Journal of the Pakistan Medical Association*.

Nemeth, E., Tuttle, M. S., *et al.* (2004) 'Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization', *Science*. doi: 10.1126/science.1104742.

Nemeth, E., Rivera, S., *et al.* (2004) 'IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin', *Journal of Clinical Investigation*. doi: 10.1172/JCI200420945.

Otto, J. M., Montgomery, H. E. and Richards, T. (2013) 'Haemoglobin concentration and mass as determinants of exercise performance and of surgical outcome', *Extreme Physiology and Medicine*. doi: 10.1186/2046-7648-2-33.

de Pascual-Teresa, S., Santos-Buelga, C. and Rivas-Gonzalo, J. G. (2000) 'Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages', *Journal of Agricultural and Food Chemistry*. doi: 10.1021/jf000549h.

Pautasso, M. (2013) 'Ten Simple Rules for Writing a Literature Review', *PLoS Computational Biology*. doi: 10.1371/journal.pcbi.1003149.

Peeling, P. *et al.* (2009) 'Training surface and intensity: Inflammation, hemolysis, and hepcidin expression', *Medicine and Science in Sports and Exercise*. doi:

10.1249/MSS.0b013e318192ce58.

Peri, L. *et al.* (2005) 'Apples increase nitric oxide production by human saliva at the acidic pH of the stomach: A new biological function for polyphenols with a catechol group?', *Free Radical Biology and Medicine*. doi: 10.1016/j.freeradbiomed.2005.04.021.

Polagruto, J. A. *et al.* (2004) ' Effects of Flavonoid-Rich Beverages on Prostacyclin Synthesis in Humans and Human Aortic Endothelial Cells: Association with Ex Vivo Platelet Function ', *Journal of Medicinal Food*. doi: 10.1089/109662003772519840.

Reardon, T. F. and Allen, D. G. (2009) 'Iron injections in mice increase skeletal muscle iron content, induce oxidative stress and reduce exercise performance', *Experimental Physiology*. doi: 10.1113/expphysiol.2008.046045.

Reeder, B. J. and Wilson, M. T. (2001) 'The effects of pH on the mechanism of hydrogen peroxide and lipid hydroperoxide consumption by myoglobin: A role for the protonated ferryl species', *Free Radical Biology and Medicine*. doi: 10.1016/S0891-5849(01)00534-2.

Reinke, S. *et al.* (2012) 'Absolute and functional iron deficiency in professional athletes during training and recovery', *International Journal of Cardiology*. doi: 10.1016/j.ijcard.2010.10.139.

Roelofs, E. J. *et al.* (2017) 'Effects of pomegranate extract on blood flow and vessel diameter after high-intensity exercise in young, healthy adults', *European Journal of Sport Science*. doi: 10.1080/17461391.2016.1230892.

Seeram, N. P. *et al.* (2008) 'Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States', *Journal of Agricultural and Food Chemistry*. doi: 10.1021/jf073035s.

Seeram, N. P., Lee, R. and Heber, D. (2004) 'Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (Punica granatum L.) juice', *Clinica*

Chimica Acta. doi: 10.1016/j.cccn.2004.04.029.

Seeram, N. P. and Nair, M. G. (2002) 'Inhibition of lipid peroxidation and structure-activity-related studies of the dietary constituents anthocyanins, anthocyanidins, and catechins', *Journal of Agricultural and Food Chemistry*. doi: 10.1021/jf025671q.

Shema-Didi, L. *et al.* (2013) 'Pomegranate juice intake attenuates the increase in oxidative stress induced by intravenous iron during hemodialysis', *Nutrition Research*. doi: 10.1016/j.nutres.2013.04.004.

Shukla, M. *et al.* (2008) 'Consumption of hydrolyzable tannins-rich pomegranate extract suppresses inflammation and joint damage in rheumatoid arthritis', *Nutrition*. doi: 10.1016/j.nut.2008.03.013.

Sukumaran, A. *et al.* (2012) 'Effects of acute and chronic inflammation on proteins involved in duodenal iron absorption in mice: A time-course study', *British Journal of Nutrition*. doi: 10.1017/S0007114512000189.

Sumaiya, K., Jahurul, M. H. A. and Zzaman, W. (2018) 'Evaluation of biochemical and bioactive properties of native and imported pomegranate (Punica granatum l.) cultivars found in Bangladesh', *International Food Research Journal*.

Sumner, M. D. *et al.* (2005) 'Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease', *American Journal of Cardiology*. doi: 10.1016/j.amjcard.2005.05.026.

Terao, J., Piskula, M. and Yao, Q. (1994) 'Protective Effect of Epicatechin, Epicatechin Gallate, and Quercetin on Lipid Peroxidation in Phospholipid Bilayers', *Archives of Biochemistry and Biophysics*. doi: 10.1006/abbi.1994.1039.

Trexler, E. T. *et al.* (2014) 'Effects of pomegranate extract on blood flow and running time to

exhaustion', *Applied Physiology, Nutrition, and Metabolism*. doi: 10.1139/apnm-2014-0137.

Trinity, J. D. *et al.* (2014) 'Impact of polyphenol antioxidants on cycling performance and cardiovascular function', *Nutrients*. doi: 10.3390/nu6031273.

Trombold, J. R. *et al.* (2010) 'Ellagitannin consumption improves strength recovery 2-3 d after eccentric exercise', *Medicine and Science in Sports and Exercise*. doi: 10.1249/MSS.0b013e3181b64edd.

Trombold, J. R. *et al.* (2011) 'The effect of pomegranate juice supplementation on strength and soreness after eccentric exercise', *Journal of Strength and Conditioning Research*. doi: 10.1519/JSC.0b013e318220d992.

Urbaniak, A., Basta, P., Ast, K., Wołoszyn, A., Kuriańska - Wołoszyn, J., *et al.* (2018) 'The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers', *Journal of the International Society of Sports Nutrition*. doi: 10.1186/s12970-018-0241-z.

Urbaniak, A., Basta, P., Ast, K., Wołoszyn, A., Kuriańska – Wołoszyn, J., *et al.* (2018) 'The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers', *Journal of the International Society of Sports Nutrition*, 15(1), p. 35. doi: 10.1186/s12970-018-0241-z.

Walsh, N. P. and Oliver, S. J. (2016) 'Exercise, immune function and respiratory infection: An update on the influence of training and environmental stress', *Immunology and Cell Biology*. doi: 10.1038/icb.2015.99.

Ward, R. J. *et al.* (2011) 'Iron and the immune system.', *Journal of neural transmission (Vienna, Austria : 1996)*. doi: 10.1007/s00702-010-0479-3.

Yusof, A. *et al.* (2007) 'Exercise-induced hemolysis is caused by protein modification and most

evident during the early phase of an ultraendurance race', *Journal of Applied Physiology*. doi: 10.1152/japplphysiol.00580.2006.

Streszczenie rozprawy doktorskiej pt.

„Wpływ suplementacji sokiem z owocu granatowca właściwego (*Punica granatum* L.) na zdolność antyoksydacyjną osocza i gospodarkę żelazem u osób trenujących wyczynowo wioślarstwo”

Owoc granatowca właściwego (*Punica granatum* L.) znany jest z wielu korzystnych właściwości, między innymi: aktywności przeciwutleniającej, zdolności do przeciwdziałania peroksydacji lipidów, oraz ograniczenia negatywnych skutków promieniowania UV-B. Korzyści te zawdzięcza wysokiej zawartości polifenoli, a w szczególności obecności takich związków jak: antocyjany, flawonole i niektórych elagitanin.

Obciążenie organizmu sportowca, intensywnym lub długotrwałym wysiłkiem fizycznym, skutkować może zaburzeniami wewnętrznej homeostazy i w konsekwencji obniżeniem wyników sportowych. Jednym z głównych wyznaczników wydajności sportowca jest metabolizm żelaza. Żelazo uczestniczy w wielu procesach fizjologicznych takich jak transport tlenu w organiźmie czy pozyskiwanie energii. Zaburzenie jego prawidłowego poziomu może być spowodowane zarówno niedoborem w diecie, jak również stanem zapalnym będącym konsekwencją intensywnego wysiłku fizycznego. Stan zapalny w organizmie związany jest ze wzmorzoną syntezą hepcydyny – hormonu biorącego udział w degradacji ferroportyny, a zatem przeciwdziałającego migracji żelaza z jego rezerw komórkowych (np. w wątrobie i śledzionie) i zakłócającego jego absorbcję z przewodu pokarmowego. Utrzymywanie się takiego stanu negatywnie oddziałuje na poziom erytrocytów i w konsekwencji może skutkować niedokrwistością.

Chociaż dokładny mechanizm działania polifenoli nie został jak dotąd udowodniony, wiadomo, że dieta bogata w elagitaniny i antocyjany jest związana ze spadkiem skurczowego ciśnienia krwi oraz wzrostem średnicy naczyń krwionośnych, co skutkuje zwiększonym

przepływem krwi. Sugeruje to związek między dietą bogatą w owoc granatu a dostarczaniem tlenu do komórek. Dodatkowo, wysoka zawartość azotanów (NO_3^-) pozytywnie oddziałuje na transport tlenu dzięki jego konwersji do NO - silnego środka rozszerzającego naczynia krwionośne. W stanie spoczynku NO syntezowany jest głownie endogennie, jednak w warunkach niedotlenienia (które są lokalnie obecne w mięśniach podczas wysiłku) szlak wtórny, tj. NO_3-azotyn (NO_2^-) - NO, nabiera większego znaczenia. Co więcej, suplementacja polifenolami zwiększa działanie NO_3^- w diecie, nie tylko poprzez stymulowanie jego konwersji do NO, ale również poprzez ochronę NO przed uszkodzeniami wolnorodnikowymi.

Celem naukowym przedstawionej dysertacji było zbadanie wpływu soku z owocu granatowca właściwego (*Punica granatum* L.) na wybrane parametry wolnorodnikowe i gospodarkę żelazem u sportowców trenujących wyczynowo wioślarstwo.

Z dostępnych baz danych zakwalifikowano i przeanalizowano jedenaście pozycji literaturowych, które dotyczyły suplementacji owocem granatu wśród sportowców i osób aktywnych fizycznie. Pomimo, że badania różniły się nie tylko pod względem poziomu wytrenowania uczestników, obciążenia treningowego, dawki i formy zastosowanego suplementu oraz czasu interwencji, jednakże można było scharakteryzować i podsumować zaobserwowane korzyści wynikające z suplementacji owocem granatowca właściwego. Korzyści te dotyczyły: poprawy siły, wzrostu poziomu witalności, zmniejszenia odczuwalnego zmęczenia i bólu mięśni, wzrostu średnicy naczyń krwionośnych i przepływu krwi, wzrostu stężenia całkowitej pojemności antyoksydacyjnej w surowicy, ograniczenia wzrostu tętna, skurczowego ciśnienia krwi, obniżenia poziomu kinazy kreatynowej i dehydrogenazy mleczanowej, przyspieszenia regeneracji powysiłkowej, obniżenia poziomów metaloproteinaz macierzy 2 i 9, białka C-reaktywnego i dialdehydu malonowego, a także wzrost aktywności enzymów przeciwutleniających (peroksydazy

glutationowej i dysmutazy ponadtlenkowej). Wyniki tych badań dowodzą, że zastosowanie soku z granatu o udokumentowanym, wysokim potencjale antyoksydacyjnym, korzystnie oddziaływanie na organizm poddany obciążeniom wysiłkowym.

W przeprowadzonych badaniach, na grupie sportowców trenujących wyczynowo wioślarstwo, suplementacja sokiem z owocu granatowca zwiększyła całkowitą pojemność antyoksydacyjną (TAC) osocza. Parametr ten był znacznie wyższy po okresie regeneracji w grupie suplementowanej, w porównaniu z grupą otrzymującą placebo. Wzrost TAC nie miał jednak znaczącego wpływu na inne markery uwzględnione w badaniu. Przed okresem suplementacji, bezpośrednio po intensywnym wysiłku fizycznym zaobserwowałam znaczący spadek TAC w obu grupach. Wolne rodniki odpowiedzialne są za inicjacje peroksydacji wielonienasyconych kwasów tłuszczowych błon erytrocytów, a tym samym nasilenie hemolizy po wysiłku. Obserwacja ta koresponduje z odnotowanym zwiększeniem steżenia żelaza w obu grupach bezpośrednio po intensywnym wysiłku fizycznym przed rozpoczęciem suplementacji. Sam wysiłek fizyczny nie miał wpływu na zmiany stężenia TAC w osoczu, w żadnej z grup. Kwas moczowy - końcowy produkt metabolizmu puryn, będący ważnym przeciwutleniaczem w osoczu krwi, nie przyczyniał się do zmian poziomów TAC, pomimo, iż wioślarze wykazywali jego podwyższone stężenia w okresie restytucji. Zaobserwowany brak statystycznie istotnych różnic w stężeniu interleukiny 6 i żelaza po wysiłku, po zakończeniu okresu suplementacji oraz hepcydyny, mioglobiny i kinazy kreatynowej na każdym etapie badań może być wyjaśnione poprzez fakt, iż badani wioślarze charakteryzowali się wysokim poziomem wytrenowania, a zatem adaptacją do wykonowanego wysiłku fizycznego. Po interwencji sportowcy z obu grup wykazali powysiłkowy znaczny wzrost stężenia utajonej zdolności wiązania żelaza, który utrzymywał się po 24 godzinach regeneracji. Podobnie po interwencyjne zmiany w stężeniu całkowitej zdolności wiązania żelaza okazały się

być niezależne od suplementacji sokiem z owocu granatowca, gdyż parametr ten znacząco zmalał w obu grupach bezpośrednio po wysiłku fizycznym i utrzymywał się na obniżonym poziomie po 24 godzinnnym okresie regeneracji. W przeprowadzonym badaniu przed interwencją zaobserwowałam wzrost powysiłkowego stężenia rozpuszczalnych receptorów transferyny (sTfR) w grupie suplementowanej i spadek tego parametru w grupie placebo. Po zakończeniu interwencji wioślarze z grupy suplementowanej wykazywali znacznie wyższy spoczynkowy poziom sTfR niż grupa kontrolna. Zarówno suplementacja jak i wysiłek fizyczny nie miały wpływu na poziom ferrytyny w żadnej z badach grup.

Summary of doctoral dissertation on

„Effect of supplementation with pomegranate fruit juice (*Punica granatum* L.) on antioxidant capacity of plasma and iron metabolism in rowers”

Pomegranate fruit (*Punica granatum* L.) is widely known for its various beneficial properties, those include: antioxidant activity, reduction of lipid peroxidation, and protection against the unfavorable consequences of UV-B radiation exposure. The efficacy of pomegranate fruit is associated with its high polyphenols content, mostly anthocyanins, flavonols and some ellagitannins.

The observed health benefits associated with pomegranate consumption prompted many scientists to investigate the effect of its supplementation on athletes and physically active subjects, since physical exercise is one of the most stressful conditions, characterized by both acute and delayed alterations in the organism’s responses.

Physical exercise places a burden on athlete’s body and can result in the disruption of internal homeostasis and consequently affect their performance. Iron metabolism is one of the key determinants of athlete performance. Iron participates in numerous physiological processes such as oxygen transport and energy synthesis. Disruption of its normal level can be caused both by its deficiency in the diet as well as inflammation resulting from the intense physical exercise. Inflammatory response is associated with the increased synthesis of hepcidin - a hormone involved in the degradation of ferroportin, thus preventing the migration of iron from its cellular reserves (e.g. in the liver and spleen) and interfering with its gastrointestinal absorption. The persistence of this condition negatively affects the level of erythrocytes and may result in anemia.

Although the exact mechanism of polyphenols activity has not yet been proven, it is known that ellagitannins and anthocyanins rich diet is associated with a decrease in systolic blood pressure

and an increase in the blood vessels diameter, which results in increased blood flow. This consequently suggests a link between a pomegranate-rich diet and oxygen delivery to cells. Additionally, high content of nitrates (NO_3^-) has a positive effect on oxygen transport due to its conversion into NO - a strong vasodilator. In resting conditions NO is synthesized mainly endogenously by NO synthases, however, under hypoxia (which is locally present in the muscles during exercise), the secondary pathway, i.e. NO_3-nitrite (NO_2^-) - NO, gains more significance. Moreover, supplementation with polyphenols rich fruits increases the effect of NO_3^- in the diet, not only by stimulating its conversion to NO, but also by protecting it from free radicals induced damage.

The aim of my doctoral thesis was to investigate the effect of supplementation with pomegranate fruit juice (*Punica granatum* L.) on antioxidant capacity of plasma and iron metabolism in rowers.

I summarized and compared data from eleven scientific articles dedicated to the effect of pomegranate supplementation in athletes and physically active subjects. The comparison of the obtained results was not trivial due to the lack of the homogeneity in the training level of athletes and processes leading to fatigue development, exercise load, supplementation dose and period of intervention. As a result, I was able to identify some main beneficial effects of pomegranate supplementation in athletes and physically active individuals. Those include improvement of the total body strength, feeling of vitality; reduction of perceived fatigue and muscle pain; increase in blood vessels' diameter and blood flow, and serum total antioxidant capacity; reduction in creatine kinase, lactate dehydrogenase, pulse, systolic blood pressure and the rate of increase of heart rate; support in the recovery of post-training creatine kinase, lactate dehydrogenase, C-reactive protein and aspartate aminotransferase to their baseline levels; lowering levels of matrix metalloproteinase

2 and 9, C-reactive protein and malonic dialdehyde; as well as increasing the activity of antioxidant enzymes (glutathione peroxidase and superoxide dismutase). Consequently, majority of the authors recommend pomegranate supplementation in athletes and physically active individuals.

In the research conducted on the group of professional rowers, supplementation with pomegranate juice lead to an increase in total antioxidant capacity (TAC) of the plasma. This parameter was significantly higher after the recovery period in the supplemented group as compared to the control group. However, the increase in TAC had no significant effect on other markers studied. Before the supplementation period, immediately after intense exercise, a significant decrease in TAC was recorded in both groups. Free radicals are responsible for the initiation of peroxidation of polyunsaturated fatty acids of erythrocyte membranes and, consequently, enhance post-exercise hemolysis. This observation corresponds with the observed increase in iron concentration in both groups immediately after intense physical exercise and prior to intervention. Exercise by itself had no effect on changes in plasma TACs in any of the groups. Uric acid - the final product of purine metabolism, which is an important antioxidant in the blood plasma, did not contribute to changes in TAC levels, although its elevated level was observed during the recovery period. The lack of statistically significant post-intervention differences in the concentrations of interleukin 6 and iron after exercise, as well as hepcidin, myoglobin and creatine kinase at each stage of the study can be explained by the fact that rowers adapted to the intense exercise. After intervention, athletes from both groups demonstrated a significant post-exercise increase in iron binding capacity, which persisted after 24 hours of regeneration. Similarly, after intervention, changes in the concentration of total iron binding capacity were found to be independent of supplementation since this parameter significantly decreased in both groups immediately after exercise and remained at a reduced levels after a 24-hour recovery period. In the

pre-intervention period, an increase in post-exercise soluble transferrin receptor (sTfR) concentration in the supplemented group and a decrease of this parameter in the control group can be observed. After the intervention, rowers from the supplemented group showed significantly higher pre-exercise sTfR levels than the control group. Ferritin levels were not affected in any of the groups by either the supplementation or intense exercise.

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Uchwała nr 357/15

Na podstawie przepisów Ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (Dz. U. 2011, Nr 277, poz. 1634 z późn. zm.); Rozporządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz. U. Nr 47, poz.480); Ustawy z dnia 6 września 2001r. Prawo farmaceutyczne (Dz. U. 2008 Nr 45, poz. 271 z późn. zm.); Rozporządzenia Ministra Finansów z dnia 30 kwietnia 2004r. w sprawie obowiązkowego ubezpieczenia odpowiedzialności cywilnej badacza i sponsora (Dz. U. 2004 nr 101, poz. 1034 z późn. zm.); Rozporządzenia Ministra Finansów z dnia 18 maja 2005r. zmieniające rozporządzenie w sprawie obowiązkowego ubezpieczenia odpowiedzialności cywilnej badacza i sponsora (Dz. U. Nr 101, poz. 845); Rozporządzenia Ministra Zdrowia z dnia 30 kwietnia 2004r. w sprawie sposobu prowadzenia badań klinicznych z udziałem małoletnich (Dz. U. 2004 Nr 104, poz. 1108); Rozporządzenia Ministra Zdrowia z dnia 30 kwietnia 2004r. w sprawie zgłaszania niespodziewanego ciężkiego niepożądanego działania produktu leczniczego (Dz. U. Nr 104, poz. 1107); Rozporządzenie Ministra Zdrowia z dnia 15 listopada 2010 r. w sprawie wzorów wniosków przedkładanych w związku z badaniem klinicznym, wysokości opłat za złożenie wniosków oraz sprawozdania końcowego z wykonania badania klinicznego (Dz. U. 2010r. nr 222 poz. 1453, z późn. zm.); Ustawy z dnia 20 maja 2010 r. o wyrobach medycznych (Dz. U. 2010r. nr 107 poz. 679, z późn. zm.); Rozporządzenie Ministra Finansów z dnia 6 października 2010 r. w sprawie obowiązkowego ubezpieczenia odpowiedzialności cywilnej sponsora i badacza klinicznego w związku z prowadzeniem badania klinicznego wyrobów (Dz. U. 2010, Nr 194 poz. 1290);Ustawa z dnia 18 marca 2011 r. o Urzędzie Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych (Dz.U. 2011 nr 82 poz. 451);Rozporządzenie Ministra Zdrowia z dnia 2 maja 2012r. w sprawie Dobrej Praktyki Klinicznej (Dz. U. 2012, poz. 489);Rozporządzenie Ministra Zdrowia z dnia 2 maja 2012r. w sprawie wzorów dokumentów przedkładanych w związku z badaniem klinicznym produktu leczniczego oraz w sprawie wysokości i sposobu uiszczania opłat za złożenie wniosku o rozpoczęcie badania klinicznego (Dz. U. 2012, Nr 0 poz. 491); w oparciu o Deklarację Helsińską - Zasady Etycznego Postępowania w Eksperymencie Medycznym z Udziałem Ludzi.

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„Wpływ suplementacji sokiem z owocu granatowca właściwego (Punica granatum L.) na wybrane parametry wolnorodnikowe i gospodarkę żelazem u sportowców tronujących wyczynowo wioślarstwo".

Komisja wydała uchwałę o pozytywnym zaopiniowaniu tego wniosku

Zastępca Przewodniczącego Komisji
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6.	mgr Jolanta Łojko-Kołodziejczak	pielęgniarka	Pielęgniarka Oddziałowa Izby Przyjęć Pediatrii Szpitala Klinicznego im. Karola Jonschera UM w Poznaniu, ul. Szpitalna 27/33, Poznań
7.	mgr Krystyna Malinger	farmaceuta	Apteka Ginekologiczno-Położniczego Szpitala Klinicznego UM, ul. Polna 33, Poznań
8.	prof. dr hab. Andrzej Marszałek	patomorfologia	Zakład Patologii Nowotworów UM ul. Garbary 15, Poznań
9.	prof. dr hab. n. med. Maciej Owecki	choroby wewnętrzne, endokrynologia	Klinika Endokrynologii, Przemiany Materii i Chorób Wewnętrznych UM, ul.Przybyszewskiego 49, Poznań
10.	prof. dr hab. Wojciech Służewski	pediatria, neurologia dziecięca, choroby zakaźne	Klinika Chorób Zakaźnych i Neurologii Dziecięcej UM ul. Szpitalna 27/33, Poznań
11.	prof. dr hab. Robert Spaczyński	ginekologia i położnictwo	Klinika Niepłodności i Endokrynologii Rozrodu UM, ul. Polna 33, 60-535 Poznań
12.	dr med. Piotr Tomczak	onkologia kliniczna, radioterapia	Klinika Onkologii UM, ul. Szamarzewskiego 82/84, Poznań
13.	prof. dr hab. Joanna Twarowska-Hauser	psychiatria	Klinika Psychiatrii Dorosłych, Zakład Genetyki w Psychiatrii UM, ul. Szpitalna 27/33, Poznań
14.	ks. prof. dr hab. Jerzy Troska	teologia, etyka	Wydział Teologiczny UAM, ul. Wieżowa 2/4, Poznań
15.	prof. dr hab. Henryk Wysocki	choroby wewnętrzne, kardiologia	Klinika Intensywnej Terapii Kardiologicznej i Chorób Wewnętrznych UM ul. Przybyszewskiego 49, Poznań

8 październik, 2019

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OŚWIADCZENIE

Oświadczam, iż w publikacji pt. "*Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic revie*w" opublikowaną w *International Journal for Vitamin and Nutrition Research* byłam odpowiedzialna za wybranie artykułów wchodzących w skład przeglądu, analizę danych, a także przygotowanie, wysłanie oraz korektę manuskryptu.

Gorzów Wlkp. dn. 27.01.2020

Dr hab. Anna Skarpańska – Stejnborn prof. AWF
Zamiejscowy Wydział Kultury Fizycznej
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Zakład Nauk Biologicznych

OŚWIADCZENIE

Oświadczam, że w pracy Urbaniak A., Skarpańska – Stejnborn A.: Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic review. International Journal for Vitamin and Nutrition Research 2019, Posted online on 12 Sep 2019. https://doi.org/10.1024/0300-9831/a000601mój udział polegał na konsultacji merytorycznej oraz korekcie manuskryptu.

.....

podpis

8 październik, 2019

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OŚWIADCZENIE

Oświadczam, iż w publikacji pt. "*The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers*" opublikowaną w *Journal of the International Society of Sports Nutrition* byłam odpowiedzialna za przygotowanie wniosku do komisji bioetycznej o zgodę na przeprowadzenie badań, pozyskanie środków z funduszu na rozwój młodych pracowników nauki, które częściowo pokryły koszty badań, zaprojektowanie badań, interpretację wyników i przygotowanie manuskryptu.

Gorzów Wlkp. dn. 1.07.2019

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Zakład Morfologicznych Podstaw

Kultury Fizycznej, Biologii i Nauk o Zdrowiu

OŚWIADCZENIE

Oświadczam, że w pracy Urbaniak A., Basta P., Ast K., Wołoszyn A., Kuriańska – Wołoszyn J., Latour E., Skarpańska – Stejnborn A.: The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers. Journal of the International Society of Sports Nutrition 2018, 15(24): 1-9. DOI 10.1186/s12970-018-0241-z [on-line] mój udział polegał na konsultacji merytorycznej oraz korekcie manuskryptu.

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Gorzów Wlkp. dn. 1.07.2019

mgr Katarzyna Ast

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doktorantka

OŚWIADCZENIE

Oświadczam, że w pracy Urbaniak A., Basta P., Ast K., Wołoszyn A., Kuriańska – Wołoszyn J., Latour E., Skarpańska – Stejnborn A.: The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers. Journal of the International Society of Sports Nutrition 2018, 15(24): 1-9. DOI 10.1186/s12970-018-0241-z [on-line] mój udział polegał na pozyskaniu i przygotowaniu suplementów do badań oraz udział w przeprowadzeniu badań.

Katarzyna Ast

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Gorzów Wlkp. dn. 1.07.2019

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OŚWIADCZENIE

Oświadczam, że w pracy Urbaniak A., Basta P., Ast K., Wołoszyn A., Kuriańska – Wołoszyn J., Latour E., Skarpańska – Stejnborn A.: The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers. Journal of the International Society of Sports Nutrition 2018, 15(24): 1-9. DOI 10.1186/s12970-018-0241-z [on-line] mój udział polegał na przeprowadzeniu testów wysiłkowych oraz opracowaniu uzyskanych wyników.

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podpis

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Zakład Teorii i Metodyki Kultury Fizycznej

OŚWIADCZENIE

Oświadczam, że w pracy Urbaniak A., Basta P., Ast K., Wołoszyn A., Kuriańska – Wołoszyn J., Latour E., Skarpańska – Stejnborn A.: The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers. Journal of the International Society of Sports Nutrition 2018, 15(24): 1-9. DOI 10.1186/s12970-018-0241-z [on-line] mój udział polegał na opracowaniu statystycznym uzyskanych wyników badań oraz korekcie artykułu.

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podpis

Gorzów Wlkp. dn. 1.07.2019

Dr Joanna Kuriańska - Wołoszyn

Akademia Jakuba z Paradyża

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OŚWIADCZENIE

Oświadczam, że w pracy Urbaniak A., Basta P., Ast K., Wołoszyn A., Kuriańska – Wołoszyn J., Latour E., Skarpańska – Stejnborn A.: The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers. Journal of the International Society of Sports Nutrition 2018, 15(24): 1-9. DOI 10.1186/s12970-018-0241-z [on-line] mój udział polegał na konsultacji merytorycznej oraz korekcie manuskryptu..

J. Kuriańska-Wołoszyn

.....

podpis

Gorzów Wlkp. dn. 1.07.2019

Dr Arkadiusz Wołoszyn

Akademia Jakuba z Paradyża

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OŚWIADCZENIE

Oświadczam, że w pracy Urbaniak A., Basta P., Ast K., Wołoszyn A., Kuriańska – Wołoszyn J., Latour E., Skarpańska – Stejnborn A.: The impact of supplementation with pomegranate fruit (Punica granatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers. Journal of the International Society of Sports Nutrition 2018, 15(24): 1-9. DOI 10.1186/s12970-018-0241-z [on-line] mój udział polegał na konsultacji merytorycznej oraz korekcie manuskryptu.

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podpis

Review

Effect of pomegranate fruit supplementation on performance and various markers in athletes and active subjects: a systematic review

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Abstract: The aim of the study was to review recent findings on the use of POM supplements in athletes of various disciplines and physically active participants. Eleven articles published between 2010 and 2018 were included, where the total number of investigated subjects was 176. Male participants constituted the majority of the group (n = 155), as compared to females (n = 21). 45% of research described was conducted on athletes, whereas the remaining studies were based on highly active participants. Randomised, crossover, double-blind study designs constituted the majority of the experimental designs used. POM supplementation varied in terms of form (pills/juice), dosage (50 ml–500 ml) and time of intervention (7 days–2 months) between studies. Among the reviewed articles, POM supplementation had an effect on the improvement of the following: whole body strength; feeling of vitality; acute and delayed muscle fatigue and soreness; increase in vessel diameter; blood flow and serum level of TAC; reduction in the rate of increase for HR, SBP, CK and LDH; support in the recovery of post-training CK, LDH, CRP and ASAT to their baseline levels; reduction of MMP2, MMP9, hsCRP and MDA; and increased activity of antioxidant enzymes (glutathione peroxidase and superoxide dismutase). In the majority of reviewed articles POM supplementation had a positive effect on a variety of parameters studied and the authors recommended it as a supplement for athletes and physically active bodies.

Keywords: punicaceae, sports, exercise

Introduction

Pomegranate (POM) (*Punicagranatum* L.) is a fleshy, red, seeded fruit of Middle East origin, widely known from its various beneficial properties. *In vitro* studies have shown the following: the high potential of POM peel powder to be used in the reduction of free radicals and lipid peroxidation (−65%) [1], a protective effect of POM fruit extract against the unfavourable consequences of UV-B radiation exposure [2] and the ability of POM ellagitannin-derived compounds for the prevention of oestrogen-responsive breast cancers [3]. Advantageous properties of POM have also been shown in vivo in various animal models. Some examples include antiskin tumour-promoting effects of topical application of POM fruit extract in CD-1 mice [4], inhibition of the development of atherosclerotic lesions in atherosclerotic mice [5] and reduction of cardiac fibrosis in Zucker diabetic fatty rats after six weeks treatment with punicagranatum flower extract (500 mg/kg, p.o.) [6]. Beneficial effects of POM supplementation were also reported in several clinical trials. These effects included improvement of stress-induced myocardial ischemia in patients with coronary heart disease [7] and reduction of carotid intima-media thickness and systolic blood pressure in carotid artery stenosis patients [8].

Numerous compounds are present in the composition of POM juice (POMj) including: anthocyanins [9], flavonoids [10], phenolic acids, e.g. ellagic, gallic and caffeic acids [11], catechin and epigallocatechin gallate [12], nutrient-minerals [13], aminoacids [14] and dietaryfibre (seeds) [15]. However, the efficacy of POMjis attributed mainly toits high content of polyphenols (~3.8 mg/ml) [16], mostly ellagic acid and ellagitannin (80–90%), with a smaller amount of anthocyanins (8–15%) [17]. A diet rich in ellagitannin and anthocyanins was linked with a decrease in systolic blood pressure (SBP) and an increase in vessel diameter and consequently, blood flow [18, 19]. These findings suggest a relationship between POM intake and O_2 delivery [20]. Additionally, a high content of nitrates (NO_3^-) positively influences O_2 delivery through its conversion to NO – a potent vasodilator [20]. Although, in resting conditions, NO is primarily generated endogenously by NO synthases (NOS), in hypoxic conditions (which are locally

List of abbreviations

POM	pomegranate
LDL	low-density lipoprotein cholesterol
TNFα	tumour necrosis factor α
COX-2	cyclooxygenase-2
RER	respiratory exchange ratio
GE	gross efficiency
TT	time trial
RPE	ratings of perceived exertion
Pmax	maximal power
IP	instantaneous power
RPM	velocity at maximal power
Tcore	core temperature
Tskin	skin temperature
SkBF	skin blood flow
PE	pomegranate extract
PV	peak velocity
VD	vessel diameter
BF	blood flow
DOMS	delayed onset of muscle soreness
CI	confidence intervals
PI	post ingestion
IPost	immediately post exercise
LA	lactic acid level
PA	polyphenol antioxidant
HR	heart rate
SV	stroke volume
CO	cardiac output
MAP	mean arterial pressure
SBP	systolic blood pressure
GLC	blood glucose
CRE	creatinine
ALT	altitude
ES	effect size
WBC	white blood cells
NEU	neutrophils
RBC	red blood cells
PLT	platelets
HDL-C	high-density lipoprotein cholesterol
Hct	haematocrit
hs-CRP	high sensitivity C-reactive protein
MMP2	matrix metalloproteinase 2
MMP9	matrix metalloproteinase 9
GPX	glutathione peroxidase
SOD	superoxide dismutase
TAC	total antioxidant capacity
MDA	malondialdehyde
CK	creatine kinase
ASAT	aspartate aminotransferase
PAL	alkaline phosphate
CRP	C-reactive protein
CAT	catalase
UA	uric acid
Tbil	total bilirubin
UIBC	Unsaturated iron binding capacity
TIBC	total iron binding capacity

present in muscles during exercise) a secondary pathway, i.e. NO_3^-nitrite (NO_2^-)-NO, gains more significance [21, 22]. Furthermore, supplementation with polyphenols enhances the effect of dietary NO_3^-, not only by promoting its conversion to NO [23, 24], but also by protecting NO from free radical-induced damage [21, 25].

Recent studies indicated that POM based supplements have a significant influence on improvement in aerobic exercise through an enhancement of the coordination of vascular O_2 delivery to muscular requirements [18, 25].

The additional advantage of supplementation with polyphenol-rich fruits is their ability to boost antioxidant potential by contributing to the increase of total antioxidant capacity (TAC) and therefore attenuate the inflammatory response (triggered e.g. during intense exercise) [26]. All of the above mechanisms of POM activity have been summarised and presented as Figure 1.

The detailed mechanisms behind the biological activity of polyphenols are not fully understood, yet their beneficial influence has certainly been proven, particularly in groups of people subjected to stressful situations [4, 8, 27, 28]. For this reason, the observed health benefits of POMj on people under stress prompted many researchers to perform studies on the association of the effect of POM supplementation with physical exercise, as this represents one of the most stressful situations, characterised by both acute and delayed alterations in the organism's responses [29].

POM fruit consumption has gained significant interest from researchers in recent years, due to its numerous benefits on the human body. This can be observed by the increasing number of scientific articles being published that are dedicated to this topic (Figure 2).

Between the years 1947–2017, a total of 2293 scientific articles related to POM were published (based on searching criteria: "pomegranate" in the title. Only articles indexed in Web of Science were included) (Figure 2). Among those, 742 were related to food science technology and 244 to nutrition dietetics (Figure 3).

Since a variety of different parameters were investigated on a wide range of athletes and intensively trained subjects, we found the need to summarise and compare the data, where possible, to gain a better understanding of the current achievements in POM-based supplementation. In this review, we have summarised the outcomes from selected research articles dedicated to the impact of POM based supplements on athletes and intensively trained subjects.

Methodology

The systematic review was designed following the guidelines of Pautasso [30]. Results have been presented in agreement with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Articles were retrieved in accordance with an extensive search employing the Web of Science database. The following search entries were used: "pomegranate supplementation", "pomegranate exercise", "pomegranate sport" or "Punicagranatum L. supplementation". References cited in selected articles

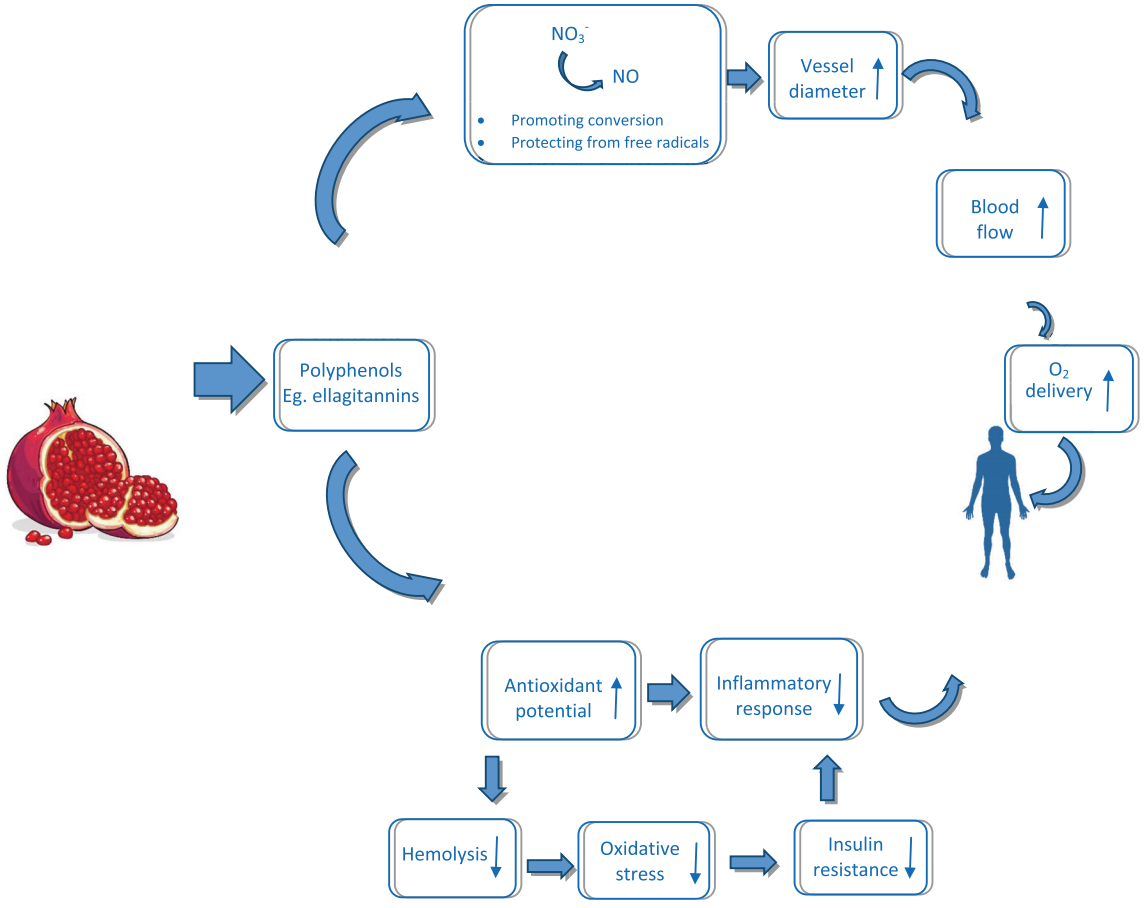

Figure 1. Summary of the mechanisms of POM activity on sport performance.

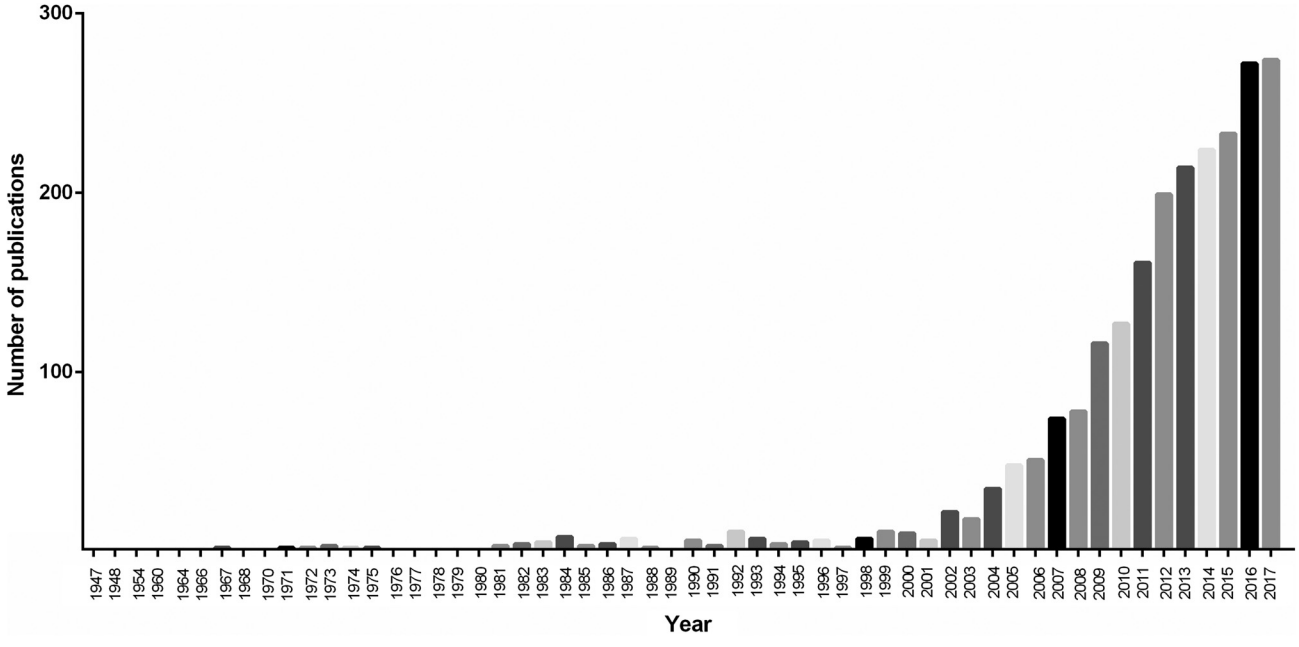

Figure 2. Number of articles published in years 1947–2017, based on the following search criteria: the word "pomegranate" in title. Only publications indexed in Web of Science were included [Accessed: January 1, 2018].

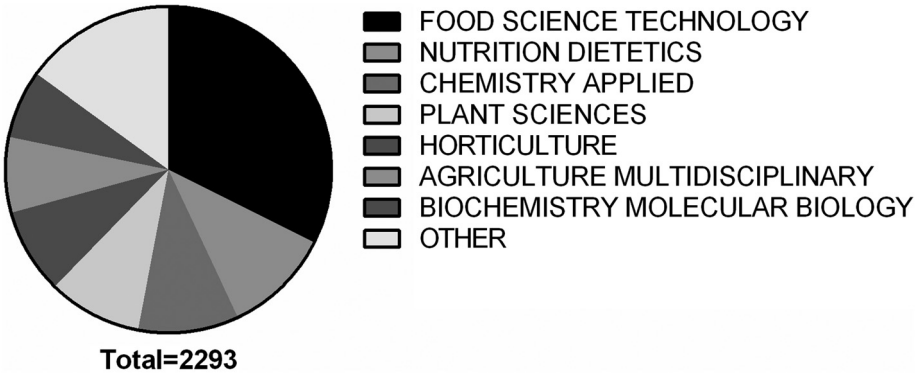

Figure 3. The distribution of articles including "pomegranate" in title between different scientific fields. Only publications indexed in Web of Science were included [Accessed: January 1, 2018].

were also considered for inclusion (n = 4). In order to ensure the purpose of the presented review, the following exclusion criteria were employed: date of publication (before 2000), language (only articles written in English were included), type of publication (meeting abstracts, letters, and reviews were excluded) and type of study (only clinical trials related to the impact of pomegranate supplements on intensively trained human subjects were included). Unpublished studies, dissertations and theses were not considered. A flow diagram representing the selection process for the systematic review has been presented in Figure 4.

Fifty-eight articles were initially identified through the database search and an additional four through literature references. Fifty-three manuscripts were selected after duplicates were removed. Of those, eighteen were excluded based on the type of article, seventeen didn't involve human subjects, six did not investigate the impact of exercise and five didn't discuss the exclusive effect of POM supplementation. Eleven articles qualified for the presented review. A further PubMed search didn't lead to the identification of any additional articles matching the selection criteria. Both authors of this review discussed and analysed articles in an independent, blinded fashion. A brief summary including sports discipline, supplement dose, group number, age of participants, gender and outcome from selected articles has been presented in Table 1.

Results

The impact factor of the journals in which articles included in this review were published varied from 0.718 to 4.291,

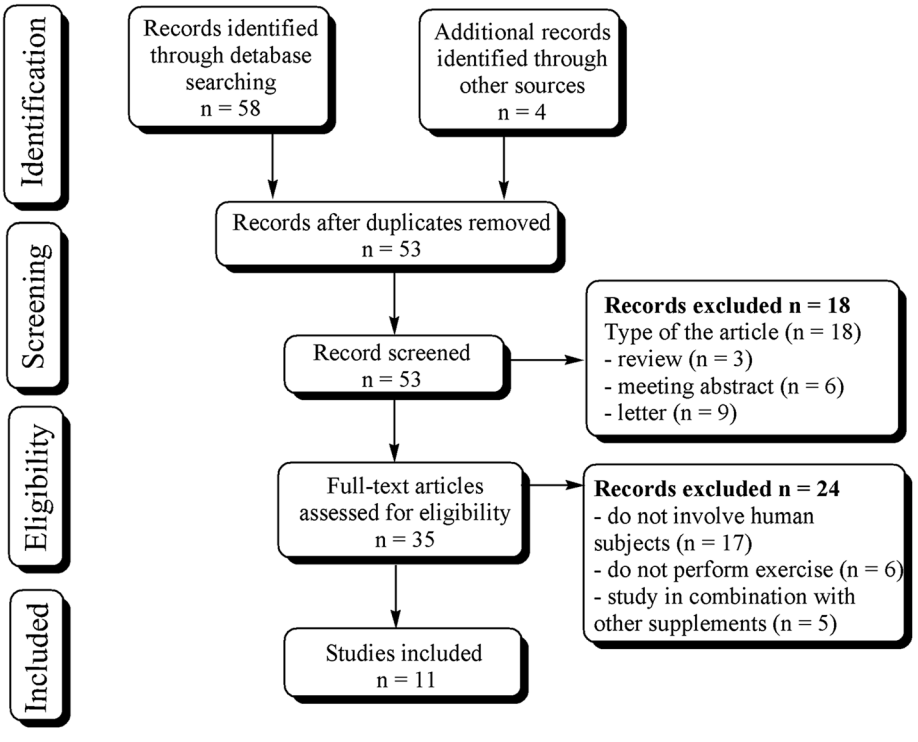

Figure 4. Flow chart of the inclusion/exclusion process of the systematic review. Search was conducted based on Web of Science. Only articles in English, published between 2000 and October 2018, were considered.

Table 1. Summary of studies investigating the effects of pomegranate supplementation on athletes and physically active subjects.

Sport discipline/ physical activity	Intervention	Number of subjects	Type of study	Age (years)†	Gender	Results	Reference
Recreational activity	POMj/PLA 500 ml/2 × per day at 12 h intervals for 9 days	16	Randomized, PLA-controlled crossover design, double-blind (14 days washout)	24.2 ± 1.4	M	**Effect of POM supplementation on physical performance, respiratory and thermoregulatory responses, the rating of perceived exertion and delayed onset soreness** Improve in the strength recovery ↑ 2–3 days after the exercise comparing with PLA: 48 h (85.4% ± 2.5% and 78.3% ± 2.6%)* and 72 h (88.9% ± 2.0% and 84.0% ± 2.0%)* after exercise; decreased soreness ↓ in POM group vs PLA 2 h after exercise* **Effect of POM on muscle damage parameters, oxidative stress and inflammatory markers** No influence on CK, myoglobin, IL-6 and CRP during recovery	31
Resistance trained subjects	POMj/PLA 250 ml/2 × per day at 12 h intervals for 15 days	17	Randomized, PLA-controlled crossover design, double-blind (14 days washout)	21.9 ± 2.4	M	**Effect of POM supplementation on physical performance, respiratory and thermoregulatory responses, the rating of perceived exertion and delayed onset soreness** Improved elbow flexion strength ↑ during 2–168 h post-exercise compared to PLA group (main treatment effect 93.6 vs. 88.9%)*; reduced elbow flexor muscle soreness ↓ for 48 and 72 h post exercise POMj vs. PLA (2.71 ± 2.11 vs. 3.77 ± 1.68 and 1.65 ± 2.09 vs. 2.41 ± 1.70) *; no influence on isometric strength and muscle soreness in the knee extensors	32
Endurance trained athletes	PA/PLA/7 days	12	Randomized, PLA-controlled crossover design, double-blind	26.8 ± 5.0	M	**Effect of POM supplementation on physical performance, respiratory and thermoregulatory responses, the rating of perceived exertion and delayed onset soreness** No effect difference between PA and PLA in performance as measured by 10 min TT following 50 min of moderate intensity cycling. Similarly no effect observed on gross efficiency, thermoregulatory responses, maximal neuromuscular power, RPE, and time to fatigue at maximal oxygen consumption at second day of testing. **Effect of POM on biological parameters and cardiovascular responses** No effect observed on LA and cardiovascular responses to exercise on either day of exercise testing.	33
Exhaustive exercised subjects	POMj/tap water 240 ml per day for 14 days	28	Randomized, double-blind, PLA-controlled	18–24	M	**Effect of POM on muscle damage parameters, oxidative stress and inflammatory markers** Post-exercise significant increase in GPX ↑*, SOD ↑* and serum levels of TAC ↑* in POMj group. Significant decrease in MMP2 ↓* and MMP9 ↓*, ceruloplasmin↓*, hs-CRP ↓* and MDA ↓* in POMj group.	36
Highly active subjects	PE/PLA 2 × 500 mg followed by 6 ounces of water 30 minutes prior to CV test	19	Randomized, crossover, double-blind, PLA-controlled crossover (7 days washout)	22 ± 2.2	10 M 9 F	**Effect of POM supplementation on physical performance, respiratory and thermoregulatory responses, the rating of perceived exertion and delayed onset soreness** Significant increase in blood flow ↑ 30 min PI with PE (BF = 40.6 ± 24.8 ml/min) comparing to PLA (BF = 29.6 ± 24.9 ml/min)*. Enlargement of vessel diameter ↑ 30 min PEx in PE vs. PLA (VD = 0.42 ± 0.07 cm vs.	25

(Continued on next page)

Table 1. (Continued)

Sport discipline/ physical activity	Intervention	Number of subjects	Type of study	Age (years)†	Gender	Results	Reference
						VD = 0.39 ± 0.07 cm)*. Significant increase in treadmill TTE ↑ at 90% (387.9 ± 199.2 vs. 346 ± 162.5 sec)* and 100% PV ↑ (170.8 ± 66.3 vs. 159.3 ± 62.3)* with PE.	
Olympic-Weightlifting	POMj/PLA 250 ml/6 × per day with 8-h intervals between supplementation (48 h treatment time) and 500 ml 1 h before training session	9	Not-randomized, double-blind, PLA-controlled crossover (48 h washout)	21 ± 0.5	M	**Effect of POM supplementation on physical performance, respiratory and thermoregulatory responses, the rating of perceived exertion and delayed onset soreness** POMj – PLA comparison: Performance (+8.29 ± 3.8% and +3.26 ± 0.83% for total and the maximal lifted amounts respectively) ↑*; RPE (−4.37 ± 1.45%) ↓*; DOMS of knee extensors (−13.4 ± 3.84%) ↓* acute effect (3 min):core temperature (+0.42%) ↑* Pre-post training comparison for POMj supplemented trial: after 48 h recovery: oral temperature ↓* **Effect of POM on biological parameters and cardiovascular responses** POMj – PLA comparison: acute effect (3 min):SBP (−1.81%) ↓*, HR (−4.46%) ↓* Pre-post training comparison for POMj supplemented trial: acute effect:GLC (−10.59 ± 3.51%) ↓*, CRE (+6.32 ± 1.57%) ↑* after 48 h recovery:SBP (−7.97 ± 1.52%) ↓*, HR↓*, no significant changes in GLC, CRE **Effect of POM on haematological parameters** Pre-post training comparison for POMj supplemented trial: acute effect:no significant changes in WBC, NEU, RBC, PLT after 48 h recovery:NEU, RBC, PAL, CRP, WBC (+11.42 ± 3.68%) ↑* **Effect of POM on muscle damage parameters, oxidative stress and inflammatory markers** POMj – PLA comparison: acute effect (3 min):CK (−8.75%) ↓*, LDH (−1.64%) ↓*, no significant changes in ASAT, PAL, CRP after 48 h recovery: pre-training level of: CK ↓*, pre-training level of LDH ↓* Pre-post training comparison for POMj supplemented trial: acute effect: no significant changes in ASAT, PAL and CRP after 48 h recovery: ASAT (−6.77 ± 0.47%) ↓*, LDH (−7.30 ± 0.86%) ↓*, CK (−11.34 ± 1.98%) ↓*	34

(Continued on next page)

Table 1. (Continued)

Sport discipline/ physical activity	Intervention	Number of subjects	Type of study	Age (years)†	Gender	Results	Reference
Endurance-based athletes	POMj/POMj: H_2O 1:1/ 200 ml/day for 21 days	20	Randomized double-blind, PLA-controlled, multicentre	33.3 ± 9.0 (control group) 35.2 ± 8.5 (POMj group) 37.5 ± 11.4 (POMj: H_2O 1:1 group)	M	**Effect of POM on hematological parameters** Increased level of lactate ↑ (from 10.3 at day 0 to 21.2 mg/dL at day 22)* and decreased of ferritin (from 69.2 at day 0 to 55.0 ng/ mL at day 22) ↓* in POMj group. Increased level of HDL-C ↑(from 48.8 at day 0 to 51.0 mg/dL at day 22)* in POMj/H_2O 1:1 supplemented group at the end of the study. Increase in K^+↑ (from 4.1 at day 0 to 4.4 mEq/L at day 22)* in POMj group. **Effect of POM on muscle damage parameters, oxidative stress and inflammatory markers** Maintained or decreased levels of carbonyls* and MDA* in POMj and POMj/H_2O 1:1 supplemented groups respectively compared with a control.	
Recreationally resistance trained subjects	PE/PLA 1000 mg/30 min prior to the tests in visits 2 and 3 and crossed over to the opposite treatment for visits 4 and 5	19	Randomized, PLA-controlled crossover design, double-blind (7–10 days washout)	22.1 ± 1.9	8 M 11 F	**Effect of POM supplementation on physical performance, respiratory and thermoregulatory responses, the rating of perceived exertion and delayed onset soreness** Significant increase of blood flow ↑IPost RSA (mean difference [MD] = 18.49 mL · min – 1)*, and IPost and 30 min Post RTF with PE. Significant increase in vessel diameter ↑* 30 min Post RSA and resulted in a significant interaction IPost and 30 min Post RTF. Significant increase in average and peak power output ↑* in sprint 5 of the RSA with PE.	18
Highlytrained cyclists	PE/PLA 1000 mg prior each trial	8	Randomized, PLA-controlled crossover design, double-blind	17–18	7 M 1F	**Effect of POM on biological parameters and cardiovascular responses** Significant increase in VO_2 max ↑* measured at 5 min into the TTE 100% in ALTPE vs. ALTPLA (+3.8 $ml.min^{-1}$ kg^{-1}, 95% CI), but no changes in SEAPOM vs. SEAPLA. No impact of PE on submaximal VO_2 values. Reduction in TTE 100% performance in ALT, but not PE. 10.3 μM greater plasma NO_3^- in PE vs. PLA (95% CI) ↑*.	20
Olympic-Weightlifting	POMj/PLA 250 ml 3 × per day during 48 h proceeding 2 training sessions + 500 ml POMj/PLA 60 min before training sessions	9	Not-randomized, double-blind, PLA-controlled crossover (48 h washout)	21.0 ± 1	M	**Effect of POM on muscle damage parameters, oxidative stress and inflammatory markers** Attenuated increase in MDA (–12.5%) ↓*, enhanced enzymatic (+8.6% for CAT and +6.8 %for GPX) ↑* and non-enzymatic (+12.6% for UA and +5.7% for Tbil)↑* antioxidant responses shortly (3 min) after training session POMj vs. PLA. Accelerated recovery kinetics of MDA (5.6%) ↑* and the enzymatic antioxidant defenses during 48 h recovery POMj vs. PLA.	37
Rowers	POMj/PLA 50 ml/day/2 months	19	Randomized, double-blind, PLA-controlled	20.8 ± 0.86 (POMj) 20.9 ± 0.95 (PLA)	M	**Effect of POM on muscle damage parameters, oxidative stress and inflammatory markers** Increased TAC (2.49 ± 0.39 vs. 1.88 ± 0.45 mmol/l) ↑* level in resting period POMj vs. PLA. Significant post-exercise increase in the concentrations of soluble transferrin receptors ↑*, iron ↑* and IL-6 ↑*. Significant post-exercise decrease in TAC ↓*. Significant increase in IL-6 ↑* 24 h post-exercise.	26

Where: † – data presented as average ± SD; ↑ – increase; ↓ – decrease; * – statistically significant; ALT – altitude; ASAT – aspartate aminotransferase; BF – blood flow; CAT – catalase; CI – confidence interval; CK – creatine kinase; CRE – creatinine; CRP – C-reactive protein; d – day; CV – critical velocity; DOMS – delayed onset of muscle soreness; F – female; GLC – blood glucose; GPX – glutathione peroxidase; HDL-C – high-density lipoprotein cholesterol; HR – heart rate; hs-CRP – high sensitivity C-reactive protein; IL-6 – interleukin – 6; IPost – immediately post exercise; LA– lactic acid level; LDH – lactate dehydrogenase; M – male; MDA – serum malondialdehyde; MMP2 – matrix metalloproteinase 2; MMP9 – matrix metalloproteinase 9; n/a – data not available; NEU – neutrophils; PA – polyphenol antioxidant; PAL – alkaline phosphate; PE – pomegranate extract; PEx – post exercise; PI – post-ingestion; PLA – placebo; PLT – platelets; POMj – pomegranate juice; PV – peak velocity; RBC – red blood cells; RPE – rating of perceived exertion; RPE – ratings of perceived exertion; RSA – repeated sprint ability; RTF – repetitions to fatigue; SBP – systolic blood pressure; SOD – superoxide dismutase; TAC – total antioxidant capacity; Tbil – total bilirubin; TIBC – total iron binding capacity; TT – time trial; TTE – time to exhaustion; UA – uric acid; UIBC – unsaturated iron binding capacity; WBC – white blood cells.

with 82% being higher than 2.500. All of them were issued between 2010 and 2018. The research analysed in this review has been summarised below by the outcome of interest.

Population characteristics

A total of 176 subjects were involved in the 11 research studies included in this review. The mean size of groups investigated was 16 ± 6 subjects. Male participants constituted the majority of investigated groups (n = 155), as compared to females (n = 21). 45% of the research described was conducted on athletes and the remaining studies on were conducted on highly active participants.

Effect of POM supplementation on physical performance, respiratory and thermoregulatory responses, the rating of perceived exertion and delayed onset soreness

Trombold et al. showed no difference in eccentric work performed by both POMj supplemented vs. PLA groups(individuals in the PLA groups consumed a drink composed of 4 g carbohydrates, specifically maltodextrin and sucralose, with the addition of colouring and flavouring substances in order to blind the treatment) [31]. In the same study, strength was significantly reduced after 2 h from eccentric exercise to 71.5% ± 7.3% and 72.8% ± 10.0%, respectively, irrespective of the treatment [31]. However, a significant improvement in strength at 48 h was observed in both groups following eccentric exercise, with a more rapid recovery from 24 h to 48 h in POMj supplemented subjects. This resulted in a higher strength in the POMj vs PLA group (85.4% ± 10.1% vs 78.3% ± 10.1%) after 48 h and 72 h (88.9% ± 7.9% vs 84.0% ± 7.8%) [31]. Moreover, the strength decrease from BASELINE to 48 h was significantly less in the POMj group (14.6% ± 10.4%) compared with the PLA group (21.7% ± 10.1%), with a concomitant improved recovery of isometric strength in POMj subjects: comparison of the rate of recovery from 2 to 48 h (PLA = 5.5%, 95% CI = 2.38%–8.68%; POMj = 13.9%, 95% CI = 7.84%–19.9%) and from 2 to 72 h (PLA = 11.3%, 95% CI = 7.98%–14.52%; POMj = 17.4%, 95% CI = 12.13%–22.59%) [31]. The same authors reported decreased soreness after 2 h from exercise in the POMj supplemented group vs. PLA (Table 1) [31].

In another study Trombold et al. reported significantly greater isometric elbow flexion strength in thePOMj supplemented group that received 250 ml/2 × d at 12 h intervals for 15 days (main treatment effect 93.6% vs. 88.9%) vs. PLA, during the 2–168 hour period after eccentric exercise [32]. The PLA juice for this study consisted of 35 g of carbohydrates: maltodextrin and sucralose with the addition of colouring and flavouring substances to resemble POMj. In the same research, isometric strength was significantly reduced at 2 h in both groups [32]. POMj supplementation had a positive effect on soreness of the elbow flexor muscles, which was significantly reduced in this group compared with the PLA treatment group between 2–168 h post-eccentric exercise [32]. Moreover, the authors reported significant treatment × time interaction and reduced elbow flexor soreness in the POMj supplemented group vs PLA at 48 and 72 h (2.71 ± 2.11 vs. 3.77 ± 1.68 and 1.65 ± 2.09 vs. 2.41 ± 1.70, respectively) [32]. The same authors reported no significant differences in isometric strength and muscle soreness in the knee extensors in POMj vs. PLA subjects (Table 1) [32].

In the study reported by Trinity et al., no significant differences in the body mass of endurance-trained athletes (polyphenol antioxidant (PA) vs. PLA groups) were observed on day 1, prior to and after a 1-hour exercise bout [33]. Similarly, as in the above studies, PLA consisted of juice containing 4 g of carbohydrates, namely maltodextrin and sucralose, with the addition of colouring and flavouring substances. Moreover, the respiratory exchange ratio (RER) and gross efficiency (GE) during cycling remained unchanged with PA supplementation [33]. The same authors observed no difference between trials in average power output during the 10 min time trial (TT), ratings of perceived exertion (RPE) at min 30 and min 45 as well as in time to fatigue [33]. Similarly, measures of maximal power (Pmax), instantaneous power (IP) and velocity at maximal power (RPM) remained unchanged between day 1 and 2 of treatments [33]. Moreover, no pair-wise differences between treatments were observed for core temperature (Tcore), skin temperature (Tskin) or estimated skin blood flow (SkBF) during the 1 h bout of exercise (Table 1) [33].

Trexler et al. found 2 × 500 mg of pomegranate extract (PE) 30 min before the exercise had a beneficial impact on the augmentation of time to exhaustion (TTE) at 90% (387.9 ± 199.2 vs. 346 ± 162.5 sec) and 100% PV (170.8 ± 66.3 vs. 159.3 ± 62.3) [25]. The same authors reported no significant change in the visual analogue (pain) scale depending on treatment. However, on the vitality scale as indicated by the following statement: "At this moment I feel alive and vital", vitality was found to be significantly higher 30 min after PE consumption as compared with PLA (capsules containing 95% maltodextrin, 5% purple carrot and hibiscus for colour) [25]. Moreover, vessel diameter (VD) was significantly increased 30 min after ingestion of PE

(VD = 0.42 ± 0.07 cm (PE) vs. VD = 0.39 ± 0.07 cm (PLA)) [25]. Similarly, blood flow (BF) was reported to be significantly elevated 30 min after PE consumption (BF = 40.6 ± 24.8 ml/min) vs. PLA (BF = 29.6 ± 24.9 ml/min) (Table 1) [25].

Ammar et al. showed supplementation with 250 ml of POMj/6 × per day with 8 h intervals between ingestion (48 h treatment time) and 500 ml 1 h before the training session improved the performance of Olympic weightlifters by 8.29 ± 3.8% and 3.26 ± 0.83%, for total and maximal lifted amounts, respectively, as compared to the PLA group [34]. As PLA, a pomegranate-flavoured commercially-available drink consisting of water, citric acid, natural flavour and identical natural flavour (pomegranate), sweeteners (aspartame × (0.3 g/l), acesulfame K (0.16 g/l), stabilisers (Arabic gum), but without antioxidants, vitamins and polyphenols, was administered. In the same study ratings of perceived exertion (RPE) and delayed onset of muscle soreness (DOMS) values were significantly influenced by POMj supplementation, showing a RPE (−4.37 ± 1.45%) and DOMS of knee extensors (48 h after training session) (−13.4 ± 3.84%), which was higher than delayed soreness of elbow flexors (i.e., 74.4 ± 4.4% vs 32.2 ± 3.6% and 61.1 ± 5.1% vs 25.5 ± 3.7%) (Table 1) [34].

Roelofs et al. reported a positive impact of 1000 mg PE consumption on average power in sprinting tests, with power significantly higher on sprint 5 (out of 10 sprints) when 95% confidence intervals (CI) were used [18]. Similarly, a significant increase in vessel diameter 30 min post ingestion (30 min PI) was reported for the PE group (administered capsules containing 95% maltodextrin, 5% purple carrot and hibiscus for colour) [18]. The same authors observed pomegranate extract consumption led to a greater baseline blood flow as compared to 30 min PI; 30 minutes post exercise (30 min Post) greater than 30 min PI; and immediately post-exercise (IPost) greater than baseline, 30 min PI, and 30 min Post [18]. The 95% CI indicated a significant difference in blood flow IPost in the 1000 mg PE supplemented group [18]. Although, the authors did not report any significant differences for repetitions completed for bench press or leg press in PE vs. PL groups, both bench press (MD = 0.63 reps) and leg press repetitions (MD = 1.9 reps) were higher in the PE group than PL [18]. In the same article, a significantly greater vessel diameter was observed in the PE group for IPostBench (MD = 0.029 cm), IPostLeg (MD = 0.042 cm) and 30 min Post (MD = 0.027 cm) [18]. Moreover, the 95% CI indicated a significant difference in IPostLeg and 30 min Post blood flow in the PE group (Table 1) [18].

In the study conducted on a rowing team, Urbaniak et al. observed no differences between groups in power output, total row time over a 2000 m distance, or pre- and post-test LA levels (Table 1) [26].

Effect of POM on biological parameters and cardiovascular responses

Trinity et al. observed no difference between trials in LA at 5 min, 30 min and post TT [33]. Similarly, for both polyphenol antioxidant (PA) and PLA, HR, stroke volume (SV) and cardiac output (CO) responses on day 1 and 2 during the first 50 min of exercise and 10 min TT were alike [33]. In the same study, mean arterial pressure (MAP) increased during exercise, but no differences between treatments were observed (Table 1) [33].

Ammar et al. showed supplementation with 250 ml of POMj/6 × per day with 8 h intervals between supplementation (48 h treatment time) and 500 ml 1 h before the training session had a significant acute effect on the increase in core temperature (+0.42%), HR (−4.46%) and systolic blood pressure (SBP) (−1.81%) in the pre-post training sessions of Olympic weightlifters [34]. In the same study, POMj supplementation influenced pre-post training session blood glucose (GLC) (−10.59 ± 3.51%) and creatinine levels (CRE) (+6.32 ± 1.57%) [34]. Moreover, the authors observed a statistically significant decrease in oral temperature, HR and SBP from 3 min to 48 h after training sessions in both groups (POMj and PLA) (Table 1) [34].

Crum et al. observed an increase in plasma NO_3^- (+10.3 μmol, 95% CI) in PE compared with PLA (capsules of the same colour, size and shape as PE, with brown sugar) conditions [20]. In the same study, no significant effect of PE or altitude (ALT) was observed on SBP [20]. However, the authors noted the trend towards an increase in SBP with PE vs. PLA [20]. In the same study, there was no significant main effect of either PE nor altitude (ALT) × treatment interaction on VO_2, VCO_2, HR or LA [20]. However, a trend towards an increase in HR with PE was observed, but had a small effect size (ES) [20]. Importantly, a significant ALT × treatment interaction for VO_2 was reported, where PE increased VO_2 at ALT but not at sea level (SEA) [20]. Moreover, the trend towards increasing with PE at ALT was observed, with a moderate effect size (Table 1) [20].

Effect of POM on haematological parameters

No acute significant change was observed by Ammar et al. in white blood cells (WBC), neutrophils (NEU), red blood cells (RBC) and platelets (PLT) in pre-post sessions of Olympic weightlifters after supplementation with 250 ml of POMj (6 × day with 8 h intervals between supplementation (48 h treatment time) and 500 ml 1 h before training sessions) [34]. However, all of those parameters significantly changed in pre-post session in PLA conditions, with lower post-training values for WBC, NEU and RBC

(−11.36 ± 3.66%; −7.97 ± 4.11% and −10.34 ± 2.02%, respectively) as well as being increased in post-training PLT (+24.97 ± 9.95%) [34]. In both conditions, a significant delayed difference was observed in pre-training values for NEU and PLT, where lower pre-training values were recorded for NEU and higher values for PLT in POMj conditions [34]. Significant training effects were registered for WBC, RBC and PLT in POMj conditions and a significant interaction of POMj supplementation × training session for RBC [34]. The same authors observed an increase in WBC level (+11.42 ± 3.68% using POMj) and no changes in NEU and RBC after a 48 h recovery period (Table 1) [34].

Fuster-Muñoz et al. reported a significant increase in glucose level between day 0 and 22 in controls and in the supplemented group (receiving 200 ml/day for 21 days POMj: H_2O 1:1) [35]. Contrary, in the same time interval, a statistically significant increase in lactate levels and a decrease in ferritin levels was observed only in the POMj group [35]. Moreover, the score of lactate (Δ lactate) was reported to be statistically higher in the POMj group as compared with controls [35]. In the same study the authors showed a statistically increased level of high-density lipoprotein cholesterol (HDL-C) in the POMj:H_2O group at the end of the experiment [35]. A significant increase in K^+ was only reported for POMj group at the end of the trial (Table 1) [35].

Crum et al. reported the haematocrit (Hct) was significantly increased by ALT (+1.4%, 95% CI) and decreased by pre-exercise consumption of 1000 mg of pomegranate extract (−0.76%, 95% CI), however, the authors highlighted a small effect size (Table 1) [20].

Effect of pomegranate on muscle damage parameters, oxidative stress and inflammatory markers

Mazani et al. reported a significant decrease in serum levels of biomarkers of inflammation, including: high sensitivity C-reactive protein (hs-CRP), matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9) after two weeks of 240 ml POMj daily supplementation [36]. Contrary, the blood levels of glutathione peroxidase (GPX), superoxide dismutase (SOD) and serum levels of total antioxidant capacity (TAC) were significantly increased in the POMj group after the intervention [36]. Additionally, the authors reported a significant decrease in serum malondialdehyde (MDA) (lipid peroxidation biomarker) content, in comparison to the control group [36]. In the same study post-exhaustive exercise blood levels of GPX and SOD, as well as serum levels of MDA, MMP2, MMP9, and ceruloplasmin, were significantly higher in controls (receiving a 240 ml cup of water) than in the POMj group (Table 1) [36].

Ammar et al. observed a significant acute increase in creatine kinase (CK) (−8.75%) and LDH (−1.64%) in Olympic weightlifter's pre-post training sessions after supplementation with 250 ml of POMj/6 × per day with 8 h intervals between supplementation (48 h treatment time) and 500 ml 1 h before training sessions [34]. In the same study, no acute effect of POMj supplementation was observed on aspartate aminotransferase (ASAT), alkaline phosphate (PAL) or C-reactive protein (CRP) in a pre-post training session [34]. However, those markers were altered in the PLA group in the following manner: ASAT (+16.59%), PAL (+04.51%) and CRP (+12.59%) [34]. Moreover, supplementation with POMj and PLA had a significant delayed effect on pre-training values of CK and LDH, where lower values were observed for the POMj group [34]. The same authors observed significant recovery decreases from 3 min to 48 h after a training session for CK (−11.34 ± 1.98%), LDH (−7.30 ± 0.86%) and ASAT (−6.77 ± 0.47%), whereas no difference were observed for PAL and CRP (Table 1) [34].

Fuster-Muñoz et al. reported a significant decrease in ASAT (from 29.5 to 23.5 U/L) and an increase in protein carbonyl levels (1.1 to 1.8 nmol/mg) and MDA (10.9 to 14.1 nmol/g protein) in the control group between day 0 and 22[35]. Moreover, in the same study, adecreased level of MDA was observed in both 200 ml/day for 21 days POMj: H2O 1:1 and 200 ml/day for 21 days POMj supplemented groups (Table 1) [35].

In another study conducted on the group of Olympic-Weightlifters, Ammar et al. reported MDA to be elevated during pre-post training sessions in both POMj and PLA conditions, with a lower rate of increase in POMj group vs. PLA (pomegranate-flavoured commercially available drink consisted of water, citric acid, natural flavour and identical natural flavour (pomegranate), sweeteners (aspartame × (0.3 g/l), acesulfame K (0.16 g/l), stabilisers (Arabic gum) but without antioxidants, vitamins and polyphenols) [37]. The same authors observed a similar effect for biomarkers of enzymatic (catalase (CAT) and GPX) and non-enzymatic (uric acid (UA) and total bilirubin (Tbil)) antioxidants, which increased from pre to post training sessions completed with both treatments, but rates of increase pre/post training sessions were enhanced after POMj supplementation [37]. Moreover, significant training-session and POMj effects on CAT, GPX, UA and Tbil were reported [37]. Additionally, the authors observed a significant training session × supplementation interaction for MDA, CAT, and UA [37]. However, markers of lipid peroxidation, as well as markers of antioxidant responses (CAT, GPX, UA, and Tbil), decreased significantly in both treatments from 3 min to 48 h after training sessions, with higher rates of decrease following POMj supplementation vs. PLA (i.e., Δ rate of decrease = 5.63%, 8.94%, 10.21%, 3.57% and 7.42% for MDA, CAT, GPX, UA and Tbil, respectively)

[37]. Importantly, a 48 h recovery period was reported to be sufficient to recover all parameters to the resting values in the POMj condition (Table 1) [37].

In the study conducted by Urbaniak et al. TAC was modulated by both physical exercise and POMj supplementation [26]. A post-exercise decrease was shown in the baseline measurements from both groups [26]. However, the TAC level was significantly higher in POMj group vs. PLA at the end of the follow-up post-recovery period [26]. In the same study post-recovery, UA levels at baseline were significantly higher than after the exercise regardless of the group [26]. The authors didn't observe any effect of POMj on serum hepcidin, myoglobin or CK levels [26]. A significant post-exercise increase in baseline iron level was shown for both groups [26]. Unsaturated iron binding capacity (UIBC) was reported to increase immediately after the exercise and remain elevated until the end of a recovery period, while exercise decreased the total iron binding capacity (TIBC), which was not affected by POMj [26]. The authors observed a post-exercise increase in soluble transferrin receptors (sTfR) at baseline in the POMj group, together with a significant decrease in PLA [26]. Moreover, post-intervention rowers from the POMj group were characterised by higher pre-exercise levels of sTfR than PLA [26]. Furthermore, physical-exercise increased interleukin-6 levels (IL-6) in both groups, which remained elevated until the post-recovery period (Table 1) [26].

Discussion

The aim of the present review was to compare outcomes from clinical trials using POM based supplements in athletes and physically active subjects. In some studies, differences in the outcomes were noted and these may be explained by heterogeneity in the training level of subjects, exercise load, supplementation dose and period of intervention as well as in the number of participants. However, some significant differences between POM supplemented and PLA groups have been reported, and these are summarised below.

Ammar et al. proved that supplementation with POMj during and 48 h prior to the weightlifting training session had a significant improvement on whole body strength [34]. This discovery is in good agreement with previous reports from Trombold et al., who reported 0.5 L of daily POMj consumption for 5 days preceding intense exercise led to an improved recovery of isometric strength 2–3 days after exercise [31]. In another study Trombold et al. observed that consumption of POMj before eccentric exercise and during the recovery period had a positive effect on the improvement of arm strength, but no impact on knee isometric strength [32]. These observations are in good agreement with previous findings about supplementation with other polyphenol-rich products [38]. Notably, Connolly et al. found supplementation with tart cherry juice accelerated strength recovery [38]. As described above, the beneficial effect of POMj is associated with its high content of polyphenols, mainly ellagitannins, which have been proven to possess biological activity in pathological inflammation and/or oxidative stress conditions [28, 32, 39].

Contradictory findings were reported by Trinity et al., where one-week supplementation with PA had no influence on performance during prolonged exhaustive exercise or during high-intensity exercise of a shorter duration in the heat [33]. Moreover, in the same study PA supplementation didn't have any effect on variables related with performance, such as gross cycling efficiency, LA and maximal neuromuscular power [33]. Similar findings were published by Crum et al. where no effect of POMx on performance and submaximal VO_2 was observed, despite a 10.3 μmol increase in plasma NO_3^- [20]. However, during intensive exercise, POMx was shown to allow maintenance of VO_2 at a workload prescribed to elicit 100% VO_2 at sea level [20].

Although antioxidants (such as those present in POM) were shown to improve exercise performance [40–46], there are contradictory studies showing that antioxidants had no effect [47–49], or a negative influence [50]. As a possible explanation of lack of activity of POMx supplementation on submaximal VO_2 and performance, the authors suggest the fact that athletes tend to have higher NO synthase activity [51] and elevated resting NO_2^- and NO_3^- values [20, 52]. As a consequence, the significance of the NO_2^- - O_3^- - NO pathway is lower, since there is a sufficient amount of NO_3^- present in the blood [20]. However, increased O_2 transport and energy production, related with an adaptation to training, results in the reduction of acidic and hypoxic muscular environments, therefore, decreasing NO synthase activity and increasing the importance of the NO_3^- pathway [20, 53, 54]. Moreover, as suggested by in vivo studies, the NO_3^- pathway is favourable in type II muscle fibres [55], thus NO_3^- supplementation may be more beneficial for untrained subjects, since they tend to have higher percentage of those muscle fibres [20, 56]. Still, this conclusion is based on in vivo studies and it remains unknown whether type II muscle fibres are preferentially involved in human individuals [20]. Finally, the authors suggest the lack of overall performance improvement may be related to the presence of "responders" and "non-responders" to POM supplementation [20]. However, verification of this hypothesis would require a larger cohort study [20].

Trexler et al. observed that PE supplementation increased running time to exhaustion and delayed fatigue at exercise intensities of 90% and 100% of peak velocity

[25]. Moreover, subjects consuming PE reported a significantly greater feeling of vitality vs. the PLA group [25]. The same authors demonstrated that PE supplementation had an effect on the increase in vessel diameter 30 min Post exercise and an increase in blood flow 30 min PI [25]. This observation was further supported by Roelofs et al. who reported that 1000 mg PE supplementation had a beneficial effect on blood flow and vessel diameter immediately after the RTF test as well as 30 min post RSA and RTF [18]. The authors suggest this may possibly improve exercise performance due to increased delivery of substrates and oxygen, as they observed an improvement in peak power in sprint 5 and 7 as well as average power output in sprint 5[18]. The authors suggest this effect is correlated with the high polyphenol content of POM, and thus its capability to increase bioavailability of NO (Figure 1) [18].

Moreover, POMj supplementation prior to intensive exercise and during the recovery was proved by many authors to have a beneficial effect on acute and delayed muscle fatigue and soreness [32, 33, 35]. Contradicting findings were observed in terms of recovery of muscles soreness, where Ammar et al. showed POMj supplementation to improve soreness of knee extensor muscles, but no elbow flexors 48 h post training [34]. Whereas Trombold et al. previously reported POMj supplementation to advance soreness recovery only in the arm, but not leg muscles [32].

Ammar et al. reported POMj supplementation modified the acute responses of the majority of tested parameters when compared with PLA outcomes [34]. Namely, the authors reported significant changes in pre-post training sessions for WBC, NEU, RBC, ASTAT, PAL and CRP, as well as a reduction in the rate of increase for HR, SBP, CK and LDH [34]. Moreover, the same authors noted a significant increase in CRE and decrease in GLC only using POMj [34]. In terms of delayed effects, Ammar et al. reported supplementation with POMj during a 48 h recovery period advanced the recovery kinetics of SBP, CK and LDH and increased the level of PLT [34]. Contradicting results have been previously published by Trombold et al., who reported that a daily drink of 0.5 L of POMj before the exercise for 5 days had no effect on inflammation markers (for example interleukin-6 and CRP) or muscle damage (for example CK and myoglobin) [31]. Moreover, Ammar et al. showed supplementation with POMj supported the recovery of post-training CK, LDH, CRP and ASAT to their baseline levels [34]. Mazani et al. reported two weeks POMj supplementation significantly reduced serum levels of MMP2, MMP9, hs-CRP and MDA as well as increasing the activity of antioxidant enzymes in healthy males undergoing exhaustive exercise [36]. Interestingly, Fuster-Muñoz et al. observed higher lactate and K^+ levels in the POMj group as compared to controls at the end of the study vs. day 0 [35]. Additionally, authors reported decreased levels of MDA as a result of POMj consumption [35]. Similarly, Ammar et al. observed a reduction in the immediate increase of MDA and acceleration in the delayed recovery kinetics of MDA as a consequence of POMj supplementation 48 h before and during training [37]. Furthermore, POMj consumption had a positive effect on the increase in acute antioxidant responses and increase in the recovery of the antioxidant markers [37]. In another study, Urbaniak et al. observed POMj that supplementation increased the level of TAC in rowers [26].

The beneficial effects of POM supplementation that are discussed in this review align with general findings about polyphenol rich fruits supplementation in athletes and physically active individuals [57]. For instance, Skarpańska-Stejnborn et al. found supplementation with cranberry [58], grape extract (*Vitis vinifera*) [59], chokeberry [60] and black currant [61] significantly strengthened antioxidant potential, therefore, improving the protection against oxidative processes in intensively trained rowers. In other studies, consumption of blackcurrant powder for 7 days ($\sim$ 105 mg anthocyanins) enhanced cycling time trial performance (16.1 km) [62], enhanced high-intensity intermittent running distance to exhaustion [63], had a positive effect on the reduction in fatigue index with repeated sprints in recreationally active participants [64, 65] and, in trained subjects, led to small (0.8%) improvement in repeated 4-km cycling time trial performance [66]. Kang et al. found 30 days supplementation with oligomerised lychee extract (200 mg polyphenols) enhanced running time to exhaustion at 80% heart rate maximum [67], while red grape skin extract consumed for 6 weeks ($1.17\ g \cdot day - 1$, 220 mg polyphenols) was reported to improve 50-m swimming time trial performance in recreationally active subjects [68].

Based on research results presented in this review, supplementation with POM may enhance sport performance, by mechanisms mostly related to its antioxidant and vascular effects. However, the studies are at an early stage and more detailed work is needed in order to optimise the training level of subjects and differences in the exercise load, supplementation dose and period of intervention.

Conclusion

Heterogeneity in the training level of subjects and processes leading to fatigue development, exercise load, supplementation dose and period of intervention and the number of participants in summarised studies are not uniform. Therefore, a comparison of results is not trivial. However, some major beneficial properties of POM supplementation in athletes and physically active subjects can be identified and summarised. These include improvement of whole body

strength, feeling of vitality, acute and delayed muscle fatigue and soreness, increase in vessel diameter, blood flow and serum level of TAC, reduction in the rate of increase for HR, SBP, CK and LDH, support in the recovery of post-training CK, LDH, CRP and ASAT to their baseline levels, reduction of MMP2, MMP9, hsCRP and MDA, as well as an increase in the activity of antioxidant enzymes. For this reason, POM supplementation should be recommended for athletes and physically active subjects. However, there is still a need for further research to investigate the influence of POM on athletes of other disciplines and markers which have not been studied to date.

References

1. Kelawala, N.S., & Ananthanarayan, L. (2004) Antioxidant activity of selected foodstuffs. Int J Food Sci Nutr. 55, 511–516.
2. Afaq, F., Malik, A., Syed, D., Maes, D., Matsui, M.S., & Mukhtar, H. (2005) Pomegranate Fruit Extract Modulates UV-B–mediated Phosphorylation of Mitogen-activated Protein Kinases and Activation of Nuclear Factor Kappa B in Normal Human Epidermal Keratinocytes. Photochem Photobiol. 81, 38–45.
3. Adams, L.S., Zhang, Y., Seeram, N.P., Heber, D., & Chen, S. (2010) Pomegranate ellagitannin-derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells In vitro. Cancer Prev Res. 3, 108–113.
4. Afaq, F., Saleem, M., Krueger, C.G., Reed, J.D., & Mukhtar, H. (2005) Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-κB pathways and inhibits skin tumorigenesis in CD-1 mice. Int J Cancer. 113, 423–433.
5. Aviram, M., Dornfeld, L., & Kaplan, M., et al. (2002) Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. Drugs Exp Clin Res. 28, 49–62.
6. Huang, T.H.W., Yang, Q., & Harada, M., et al. (2005) Pomegranate flower extract diminishes cardiac fibrosis in Zucker diabetic fatty rats: Modulation of cardiac endothelin-1 and nuclear factor-kappa B pathways. J Cardiovasc Pharmacol. 46, 856–862.
7. Sumner, M.D., Elliott-Eller, M., & Weidner, G., et al. (2005) Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. Am J Cardiol. 96, 810–814.
8. Aviram, M., Rosenblat, M., & Gaitini, D., et al. (2004) Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. Clin Nutr. 23, 423–433.
9. Du, C.T., Wang, P.L., & Francis, F.J. (1975) Anthocyanins of pomegranate, Punicagranatum. J Food Sci. 40, 417–418.
10. Gómez-Caravaca, A.M., Verardo, V., Toselli, M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Caboni, M.F. (2013) Determination of the major phenolic compounds in pomegranate juices by HPLC-DAD-ESI-MS. J Agric Food Chem. 61, 5328–5337.
11. Amakura, Y., Okada, M., Tsuji, S., & Tonogai, Y. (2000) Determination of phenolic acids in fruit juices by isocratic column liquid chromatography. J Chromatogr A. 891, 183–188.
12. de Pascual-Teresa, S., Santos-Buelga, C., & Rivas-Gonzalo, J. G. (2000) Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. J Agric Food Chem. 48, 5331–5337.
13. Waheed, S., Siddique, N., Rahman, A., Zaidi, J.H., & Ahmad, S. (2004) INAA for dietary assessment of essential and other trace elements in fourteen fruits harvested and consumed in Pakistan. J Radioanal Nucl Chem. 260, 523–531.
14. Lansky, E.P., & Newman, R.A. (2007) Punicagranatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J. Ethnopharmacol. 109, 177–206.
15. Sumaiya, K., Jahurul, M.H.A., & Zzaman, W. (2018) Evaluation of biochemical and bioactive properties of native and imported pomegranate (Punicagranatum l.) cultivars found in Bangladesh. Int Food Res J. 25, 737–746.
16. Seeram, N.P., Aviram, M., & Zhang, Y., et al. (2008) Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J Agric Food Chem. 56, 1415–1422.
17. Seeram, N.P., Lee, R., & Heber, D. (2004) Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (Punicagranatum L.) juice. Clin Chim Acta. 348, 63–68.
18. Roelofs, E.J., Smith-Ryan, A.E., Trexler, E.T., Hirsch, K.R., & Mock, M.G. (2017) Effects of pomegranate extract on blood flow and vessel diameter after high-intensity exercise in young, healthy adults. Eur J Sport Sci. 17, 317–325.
19. Labonté, K., Couillard, C., Motard-Bélanger, A., Paradis, M.-E., Couture, P., & Lamarche, B. (2013) Acute Effects of Polyphenols from Cranberries and Grape Seeds on Endothelial Function and Performance in Elite Athletes. Sports. 1, 55–68.
20. Crum, E.M., Che Muhamed, A.M., Barnes, M., & Stannard, S.R. (2017) The effect of acute pomegranate extract supplementation on oxygen uptake in highly trained cyclists during high-intensity exercise in a high-altitude environment. J Int Soc Sports Nutr. 14, 14.
21. Lundberg, J.O.N., Weitzberg, E., Lundberg, J.M., & Alving, K. (1994) Intragastric nitric oxide production in humans: Measurements in expelled air. Gut. 35, 1543–1546.
22. Peri, L., Pietraforte, D., Scorza, G., Napolitano, A., Fogliano, V., & Minetti, M. (2005) Apples increase nitric oxide production by human saliva at the acidic pH of the stomach: A new biological function for polyphenols with a catechol group? Free Radic Biol Med. 39, 668–681.
23. Gago, B., Lundberg, J.O., Barbosa, R.M., & Laranjinha, J. (2007) Red wine-dependent reduction of nitrite to nitric oxide in the stomach. Free Radic Biol Med. 43, 1233–1242.
24. Ignarro, L.J., Byrns, R.E., Sumi, D., de Nigris, F., & Napoli, C. (2006) Pomegranate juice protects nitric oxide against oxidative destruction and enhances the biological actions of nitric oxide. Nitric Oxide – Biol Chem. 15, 93–102.
25. Trexler, E.T., Smith-Ryan, A.E., Melvin, M.N., Roelofs, E.J., & Wingfield, H.L. (2014) Effects of pomegranate extract on blood flow and running time to exhaustion. Appl Physiol Nutr Metab. 39, 1038–1042.
26. Urbaniak, A., Basta, P., & Ast, K., et al. (2018) The impact of supplementation with pomegranate fruit (Punicagranatum L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers. J Int Soc Sports Nutr. 15, 35.
27. Shukla, M., Gupta, K., Rasheed, Z., Khan, K.A., & Haqqi, T.M. (2008) Consumption of hydrolyzable tannins-rich pomegranate extract suppresses inflammation and joint damage in rheumatoid arthritis. Nutrition. 24, 733–743.
28. Polagruto, J.A., Schramm, D.D., Wang-Polagruto, J.F., Lee, L., & Keen, C.L. (2004) Effects of Flavonoid-Rich Beverages on Prostacyclin Synthesis in Humans and Human Aortic

Endothelial Cells: Association with Ex Vivo Platelet Function. J Med Food. 6, 301–308.
29. Ammar, A., Bailey, S.J., & Chtourou, H., et al. (2018) Effects of pomegranate supplementation on exercise performance and post-exercise recovery in healthy adults: a systematic review. Br J Nutr. 120, 1201–1216.
30. Pautasso, M. (2013) Ten Simple Rules for Writing a Literature Review. PLoS Comput Biol. 9, e1003149.
31. Trombold, J.R., Barnes, J.N., Critchley, L., & Coyle, E.F. (2010) Ellagitannin consumption improves strength recovery 2–3 d after eccentric exercise. Med Sci Sports Exerc. 42, 493–498.
32. Trombold, J.R., Reinfeld, A.S., Casler, J.R., & Coyle, E.F. (2011) The effect of pomegranate juice supplementation on strength and soreness after eccentric exercise. J Strength Cond Res. 25, 1782–1788.
33. Trinity, J.D., Pahnke, M.D., Trombold, J.R., & Coyle, E.F. (2014) Impact of polyphenol antioxidants on cycling performance and cardiovascular function. Nutrients. 6, 1273–1292.
34. Ammar, A., Turki, M., & Chtourou, H., et al. (2016) Pomegranate supplementation accelerates recovery of muscle damage and soreness and inflammatory markers after a weightlifting training session. PLoS One. 11, e0160305.
35. Fuster-Muñoz, E., Roche, E., Funes, L., Martínez-Peinado, P., Sempere, J.M., & Vicente-Salar, N. (2016) Effects of pomegranate juice in circulating parameters, cytokines, and oxidative stress markers in endurance-based athletes: A randomized controlled trial. Nutrition. 32, 539–545.
36. Mazani, M., Fard, A.S., Baghi, A.N., Nemati, A., & Mogadam, R. A. (2014) Effect of pomegranate juice supplementation on matrix metalloproteinases 2 and 9 following exhaustive exercise in young healthy males. J Pak Med Assoc. 64, 785–790.
37. Ammar, A., Turki, M., & Hammouda, O., et al. (2017) Effects of pomegranate juice supplementation on oxidative stress biomarkers following weightlifting exercise. Nutrients. 9, E819.
38. Connolly, D.A.J., McHugh, M.P., & Padilla-Zakour, O.I. (2006) Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. Br J Sports Med. 40, 679–683.
39. Heber, D., Seeram, N.P., & Wyatt, H., et al. (2007) Safety and antioxidant activity of a pomegranate ellagitannin-enriched polyphenol dietary supplement in overweight individuals with increased waist size. J Agric Food Chem. 55, 10050–10054.
40. Davis, J.M., Murphy, E.A., Carmichael, M.D., & Davis, B. (2009) Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. Am J PhysiolIntegr Comp Physiol. 296, R1071–R1077.
41. MacRae, H.S.H., & Mefferd, K.M. (2006) Dietary antioxidant supplementation combined with quercetin improves cycling time trial performance. Int J Sport Nutr Exerc Metab. 16, 405–419.
42. McKenna, M.J., Bangsbo, J., & Renaud, J.-M. (2007) Muscle K^+, Na^+, and Cl^- disturbances and Na^+ -K^+ pump inactivation: implications for fatigue. J Appl Physiol. 104, 288–295.
43. McKenna, M.J., Medved, I., & Goodman, C.A., et al. (2006) N-acetylcysteine attenuates the decline in muscle Na^+, K^+-pump activity and delays fatigue during prolonged exercise in humans. J Physiol. 576, 279–288.
44. Medved, I., Brown, M.J., & Bjorksten, A.R., et al. (2004) N -acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. J Appl Physiol. 97, 1477–1485.
45. Medved, I., Brown, M.J., Bjorksten, A.R., & McKenna, M.J. (2004) Effects of intravenous N-acetylcysteine infusion on time to fatigue and potassium regulation during prolonged cycling exercise. J Appl Physiol. 96, 211–217.
46. McKenna, M.J., & Hargreaves, M. (2007) Resolving fatigue mechanisms determining exercise performance: integrative physiology at its finest!. J Appl Physiol. 104, 286–287.
47. Cheuvront, S.N., Ely, B.R., & Kenefick, R.W., et al. (2008) No effect of nutritional adenosine receptor antagonists on exercise performance in the heat. Am J Physiol Integr Comp Physiol. 296, R394–401.
48. Nieman, D.C., Henson, D.A., & Maxwell, K.R., et al. (2009) Effects of quercetin and ECGC on mitochondrial biogenesis and immunity. Med Sci Sports Exerc. 41, 1467–1475.
49. Utter, A.C., Nieman, D.C., & Kang, J., et al. (2009) Quercetin does not affect rating of perceived exertion in athletes during the Western States endurance run. Res Sport Med. 17, 71–83.
50. Gomez-Cabrera, M.C., Domenech, E., & Romagnoli, M., et al. (2008) Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr. 87, 142–149.
51. McConell, G.K., Bradley, S.J., Stephens, T.J., Canny, B.J., Kingwell, B.A., & Lee-Young, R.S. (2007) Skeletal muscle nNOS mu protein content is increased by exercise training in humans. Am J Physiol Integr Comp Physiol. 293, R821–828.
52. Vassalle, C., Lubrano, V., Domenici, C., & L'Abbate, A. (2003) Influence of chronic aerobic exercise on microcirculatory flow and nitric oxide in humans. Int J Sports Med. 24, 30–35.
53. Jensen, L., Bangsbo, J., & Hellsten, Y. (2004) Effect of high intensity training on capillarization and presence of angiogenic factors in human skeletal muscle. J Physiol. 557, 571–582.
54. Wilkerson, D.P., Hayward, G.M., Bailey, S.J., Vanhatalo, A., Blackwell, J.R., & Jones, A.M. (2012) Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. Eur J Appl Physiol. 112, 4127–4134.
55. Ferguson, S.K., Hirai, D.M., Copp, S.W., Holdsworth, C.T., Allen, J.D., & Jones, A.M., et al. (2013) Effects of nitrate supplementation via beetroot juice on contracting rat skeletal muscle microvascular oxygen pressure dynamics. Respir Physiol Neurobiol. 187, 250–255.
56. Holloszy, J.O., & Coyle, E.F. (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol. 56, 831–838.
57. Bowtell, J., & Kelly, V. (2019) Fruit-Derived Polyphenol Supplementation for Athlete Recovery and Performance. Sport. Med. 49, 3–23.
58. Skarpańska-Stejnborn, A., Basta, P., Trzeciak, J., Michalska, A., Kafkas, M.E., & Woitas-Ślubowska, D. (2017) Effects of cranberry (Vaccinum macrocarpon) supplementation on iron status and inflammatory markers in rowers. J Int Soc Sports Nutr. 14, 7.
59. Skarpańska-Stejnborn, A., Basta, P., Pilaczyńska-Szczesniak, Ł., & Horoszkiewicz-Hassan, M. (2010) Black grape extract supplementation attenuates blood oxidative stress in response to acute exercise. Biol Sport. 27, 41–46.
60. Skarpańska-Stejnborn, A., Basta, P., Sadowska, J., & Pilaczyńska-Szcześniak, Ł. (2014) Effect of supplementation with chokeberry juice on the inflammatory status and markers of iron metabolism in rowers. J Int Soc Sports Nutr. 11, 48.
61. Skarpańska-Stejnborn, A., Basta, P., & Pilaczyńska-Szcześniak, Ł. (2006) Theinfluence ofsupplementationwith the black currant (Ribes nigrum) extract on selected prooxidative-antioxidative balance parameters in rowers. Stud Phys Cult Tour. 13, 51–58.
62. Cook, M.D., Myers, S.D., Blacker, S.D., & Willems, M.E.T. (2015) New Zealand blackcurrant extract improves cycling performance and fat oxidation in cyclists. Eur J Appl Physiol. 115, 2357–2365.

63. Perkins, I.C., Vine, S.A., Blacker, S.D., & Willems, M.E.T. (2015) New Zealand blackcurrant extract improves high-intensity intermittent running. Int J Sport Nutr Exerc Metab. 25, 487–493.
64. Willems, M., Cousins, L., Williams, D., & Blacker, S. (2016) Beneficial Effects of New Zealand Blackcurrant Extract on Maximal Sprint Speed during the Loughborough Intermittent Shuttle Test. Sports. 4, E42.
65. Godwin, C., Cook, M., & Willems, M.E.T. (2017) Effect of New Zealand Blackcurrant Extract on Performance during the Running Based Anaerobic Sprint Test in Trained Youth and Recreationally Active Male Football Players. Sports. 5, E69.
66. Murphy, C., Cook, M., & Willems, M.E.T. (2017) Effect of New Zealand Blackcurrant Extract on Repeated Cycling Time Trial Performance. Sports. 5, E25.
67. Kang, S.W., Hahn, S., Kim, J.-K., Yang, S.-M., Park, B.-J., & Chul Lee, S. (2011) Oligomerized lychee fruit extract (OLFE) and a mixture of vitamin C and vitamin E for endurance capacity in a double blind randomized controlled trial. J Clin Biochem Nutr. 50, 106–113.
68. Sadowska-Kreôpa, E., Kõõlapcińska, B., Kimsa, E., & Karpiński, R. (2008) Effects of Supplementation With Red Grape Skin Polyphenolic Extract and Interval Swimming Test on the Blood Antioxidant Status in Healthy Men. Med Sport. 12, 1–7.

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The impact of supplementation with pomegranate fruit (*Punica granatum* L.) juice on selected antioxidant parameters and markers of iron metabolism in rowers

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Abstract

Background: The aim of this study was to analyse the effect of pomegranate juice (POM) supplementation on the levels of selected pro-inflammatory cytokines, hepcidin and markers of iron metabolism in well-trained rowers.

Method: The double-blind placebo-controlled study included 19 members of the Polish Rowing Team. The athletes were randomised into the supplemented group ($n = 10$), receiving 50 ml of standardised POM daily for two months, or the placebo group ($n = 9$). The subjects performed a 2000 m test on the rowing ergometer at the start of the project (baseline) and end of follow-up period. Blood samples from the antecubital vein were obtained three times during each trial: prior to the exercise, one minute after the test, and following a 24 h recovery.

Results: The study documented the beneficial effect of supplementation with pomegranate fruit juice on TAC *($P < 0.002$)*. During the resting period, TAC level in the supplemented group was significantly higher than in the placebo group ($x \pm SD$, 2.49 ± 0.39 vs. 1.88 ± 0.45, *$P < 0.05$*). The ergometric test conducted at baseline demonstrated a significant post-exercise increase in the concentrations of soluble transferrin receptors *($P < 0.04$)*, iron *($P < 0.002$)* and IL-6 *($P < 0.02$)*, and to a significant post-exercise decrease in TAC. A significant increase in IL-6 concentration was also observed 24 h post-exercise. The exercise test conducted at the end of the follow-up period resulted in a significant decrease in TBIC and a significant increase in UIBC *($P < 0.001$)*, observed in both groups, both immediately post-exercise and after the resting period.

Conclusion: Supplementation with POM contributed to a significant strengthening of plasma antioxidant potential in the group of well-trained rowers, but had no effect on iron metabolism markers.

Keywords: Pomegranate, Strenuous exercise, Training, Inflammation

Background

Physical training, in particular strenuous exercises, places a considerable burden on an athlete's body. To achieve outstanding results in competitive sports, athletes need to bear large, and not infrequently extreme, training loads. This can result in a disruption to their intrinsic homeostasis, and thus have an unfavourable effect on their performance. Published evidence suggests that a key determinant of athletes' performance is iron metabolism [1, 2]. Normal levels of iron are a prerequisite for many physiological processes, such as oxygen transport and energy synthesis [3].

The level of iron in the human body is affected not only by an adequate dietary intake of this element, but also by exercise-induced inflammation [4]. The discovery of hepcidin, a hormone, provided the link between iron deficiency and concomitant inflammation [5]. Studies have demonstrated that moderate-intensity training has a beneficial effect on iron metabolism, whereas strenuous

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exercise may induce systemic inflammation [6]. The inflammatory response is associated with enhanced synthesis of hepcidin; this hormone is involved in the degradation of ferroportin, and thus prevents the mobilisation of iron from its cellular deposits (e.g. in the liver and spleen) and interferes with its gastrointestinal absorption [7]. If this state persists, it may negatively affect erythrocyte parameters, and eventually lead to anaemia [8]. Reinke et al. [9] showed that at the end of competitive season, 70% of professional athletes (rowers and football players) presented with functional iron deficiency, and 27% had an absolute deficiency of this element. Unfavourable changes in iron parameters after strenuous exercise have also been reported by other authors [10].

Additional supplementation with iron-rich preparations does not necessarily bring expected results, and may be even harmful to an athlete's health [11, 12]. In an animal study conducted by Reardon et al. [13], injection of iron contributed to an increase in muscle and plasma concentrations of this element and to exacerbation of oxidative stress; as a result, animals from iron-supplemented group presented with lower muscle power than the controls and performed less work during the treadmill test.

The post-exercise increase in hepcidin level has been also postulated to result from an increase in the so-called labile iron pool (LIP) [1], caused by enhanced haemolysis [14]. Products of heme degradation may stimulate the generation of reactive oxygen species, leading to further damage of erythrocyte membranes. The latter are abundant in polyunsaturated fatty acids, which makes them particularly prone to oxidative injury. This self-perpetuating process may last as long as its natural "attenuation" occurs.

Pomegranate juice (POM) from the fruits of *Punnica Granatum* L. is a rich source of polyphenols, such as anthocyanins, flavanols and some ellagitannins, especially punicalagin [15]. Many studies have documented the beneficial effects of POM consumption in the treatment of various disorders [16, 17]. Researchers have recently become increasingly interested in the dietary supplementation of athletes with POM. Fuster-Mũnoz et al. [18] demonstrated that POM exerted a positive effect on the modulation of fat and protein damage in well-trained endurance-based athletes. Ammar et al. [19] showed that the consumption of POM 48 h prior to, and during training sessions contributed to the alleviation of pain, delayed damage, inflammation, and soreness of the knee flexor, accelerated the recovery kinetics of biological parameters and improved performance in nine elite weightlifters.

One group of polyphenols in pomegranate juice are anthocyanins. These compounds have an array of biological activities, showing antioxidant properties [20], acting as immunostimulants, modulating inflammatory response [21] and chelating iron ions, which may contribute to the reduction of LIP [22].

We hypothesised that supplementation with pomegranate fruit juice may boost the antioxidant potential of the athletes, contributing to an increase in TAC, and may thus attenuate the inflammatory response triggered by intense physical exercise. We also examined whether, and to what extent, these changes affected iron metabolism parameters in the study subjects.

Methods

The protocol of the study was approved by the local bioethics committee at the University of Medical Sciences in Poznan (Decision no. 357/15). All athletes were adequately informed about the nature of the study and provided their written consent to participate in the project.

Participants

The study included a group of 19 male rowers, members of the Polish National Team, who participated in an eight-week training camp between the preparatory and competitive periods. The basic characteristics of the study athletes are shown in Table 1. The study subjects were randomised to one of two groups, receiving standardised POM (supplemented group, $n = 10$) or a placebo (control group, $n = 9$).

Food intake

Rowers from the supplemented group received 50 ml of a standardised, commercially available POM (Oleofarm®) daily, for two months. The product was 100% pure natural juice squeezed from fresh fruit, with a total polyphenol content equal to 220 mg/100 g. Athletes from the non-supplemented group received the same dose of a placebo composed of water, sugar and grenadine, with a colour and taste resembling that of the pomegranate fruit juice. Both pomegranate fruit juice and placebo were provided by the same manufacturer (Oleofarm®), packed in identical dark bottles labelled with encoded information about the type of preparation and its recommended dosage. The labels were decoded at the end of the study.

The athletes completed food intake questionnaires on each day of the study period, which were later used to

Table 1 Basic characteristics of the study groups (mean ± standard deviation)

Parameter	Supplemented group ($n = 10$)	Control group ($n = 9$)
Age (years)	20.8 ± 0.86	20.9 ± 0.95
Body weight (kg)	89.4 ± 8.97	83.85 ± 12.04
Body height (cm)	192.1 ± 6.64	189.6 ± 5.79
Duration of training (years)	8.2 ± 0.78	7.14 ± 0.69

calculate the energy equivalents for their diet and the dietary intake of antioxidants and vitamins. All study subjects agreed that they refrained from drugs, medications and dietary supplements for at least two weeks preceding the study and throughout the whole study period.

Experimental procedure

The characteristics of training profiles, such as intensity, volume (in min) and type (specific, i.e. rowing: endurance, technical, speed, etc., and non-specific: jogging, strength) were recorded on a daily basis. Training intensity was classified in relation to the lactic acid (LA) threshold (4 mmol/L), as an extensive (below the LA threshold) or intensive (above the LA threshold) workload (Table 2). On the first day (prior to supplementation, at the baseline) and at the end of the training camp (after supplementation, at the end of the follow-up period), the athletes performed a controlled 2000-m rowing exercise test (Concept II, Model D, USA). All study subjects were asked to perform both tests at their maximal pace. Each test was preceded by a five minute warm-up session.

Blood samples were collected for the analysis (0.9 ml) prior to each 2000 m test (in the morning, after an overnight fasting), one minute after the test and following a 24 h recovery period. Immediately after collection, the samples were centrifuged to separate erythrocytes from serum. The serum was immediately frozen and stored at − 80 °C until the analysis. Capillary blood samples were obtained via an ear lobe prick before and after each exercise test, to assess LA levels.

Measurements

Total antioxidant capacity (TAC), was measured as an indicator of plasma antioxidant capacity with a commercially available ELISA kit (Cayman, cat no. Antioxidant Assay 709,001–96, USA); the results were expressed in mmol/L. Uric acid (UA) level was determined with a commercially available kit (Alpha Diagnostics, Cat No. K6681–100); the results were expressed in mg/dL. Serum interleukin 6 (IL-6) was quantified with a commercially available enzyme-linked immunosorbent assay (ELISA; Quantikine HS, R&D Systems, Minneapolis, USA); the results were expressed in pg/ml. Serum hepcidin was measured using a commercially available ELISA kit (Wuhan EIAab Science Co., China); the results were expressed in ng/mL. Iron concentration and TIBC were determined using colorimetric method with chromogens (cat. no. 1–418–01-50; 1–421-0060 BioMaxima, Poland); the results were expressed in μg/dL. Unsaturated iron-binding capacity (UIBC) was calculated from the formula: UIBC = TIBC − Fe. Myoglobin concentration was determined immunochemically, using Myoglobin ELISA kit (Biocom, cat. no. 11170); the results were expressed in ng/mL. Serum ferritin was quantified immunochemically, with a commercially available diagnostic kit (Demeditec, Germany); the results were expressed in ng/ml. Concentration of soluble transferrin receptor (sTfR) was determined immunochemically, with a commercially available diagnostic kit (Biocom, cat. no. RD194011100); the results were expressed in μg/mL. Creatine kinase (CK) activity in blood plasma was determined with a commercially available kit (Dr Lange, Germany, cat. no. LCN 282); the results were expressed in U/L. The coefficients of variation for all assays were < 12%.

Table 2 Training schedules during the weeks preceding blood samples before (Trial I) and after (Trial II) the supplementation

	Days before blood sample collection						
	1	2	3	4	5	6	7
Total training time [min/day]	120	100	200	190	210	150	120
Time rowed [min/day]	110	100	100	100	70	90	100
Distance rowed [km/day]	22	20	20	20	16	18	20
Training for force development [min/day]	–	–	90	–	70	–	–
Extensive endurance rowing training time [min/day]	70	100	100	40	40	90	100
High-intensity endurance rowing training time [min/day]	40	–	–	60	30	–	–
Unspecific training (running etc.) [min/day]	10	–	10	90	70	60	20
Total training time [min/day]	180	100	190	160	200	90	130
Time rowed [min/day]	160	100	130	140	120	90	125
Distance rowed [km/day]	32	18	26	28	20	16	20
Training for force development [min/day]	–	–	60	–	60	–	–
Extensive endurance rowing training time [min/day]	160	100	130	90	94	90	125
High-intensity endurance rowing training time [min/day]	–	–	–	30	26	–	5
Unspecific training (running etc.) [min/day]	20	–	25	20	20	–	–

The concentration of LA in capillary blood was measured immediately after sampling, with a commercially available kit (cat. no. LKM 140, HACH LANGE, Düsseldorf, Germany); the results were presented in mmol/L.

Statistical analysis

Statistical analysis of the results was carried out with the STATISTICA v. 10.0 software package (StatSoft, Cracow, Poland). The significance of intergroup and intragroup differences was verified using 2 (supplemented and placebo group) × 3 (timing of measurement) repeated measures analysis of variance (ANOVA). Normal distribution of the study variables was verified with a Shapiro-Wilk test. When statistically significant differences were documented on ANOVA, Fisher's *post-hoc* tests were conducted to identify the source of variance. The anthropometric characteristics of the study groups were compared with unpaired Student *t*-tests. Except for the rowing time, the results of the 2000 m tests performed prior to and after the supplementation period were subjected to intragroup and intergroup comparisons with paired and unpaired Student *t*-tests, respectively. The results of the 2000 m simulated rowing test were subjected to one-way ANOVA. The statistical characteristics of the study variables are presented as arithmetic means ± standard deviations (SD). The threshold of statistical significance for all tests was set at $p < 0.05$.

Results

Athletes from the supplemented group did not differ significantly from the controls in terms of their anthropometric parameters, age and training experience (Table 1). No statistically significant intergroup differences were found in power output, total row time over a 2000 m distance, or pre- and post-test LA levels (Table 3). The TAC values were determined prior to and after the supplementation period, and are presented in Fig. 1a. TAC turned out to be modulated by both physical exercise ($p < 0.029$) and POM supplementation ($p < 0.002$). In the baseline measurements, athletes from both study groups showed a post-exercise decrease in TAC levels. At the end of the follow-up, post-recovery TAC level in athletes from the supplemented group was significantly higher than in the controls. Regardless of the study group, post-recovery UA concentrations at baseline were significantly higher ($p < 0.0004$) than those determined immediately after the exercise test (Fig. 1b). Supplementation with POM had no significant effects on serum hepcidin, myoglobin or creatine kinase levels (Fig. 2a, b, d). Athletes from both groups showed a significant post-exercise increase in iron level ($p < 0.002$, Fig. 2c) at baseline measurements. Statistically significant changes in UIBC and TIBC were observed in both study groups, but were statistically significant post-intervention (Fig. 3a and b respectively). UIBC increased immediately after the exercise test ($p < 0.001$) and remained elevated until the end of the recovery period. The exercise test contributed to a significant decrease in TIBC level ($p < 0.001$), which persisted at the end of the recovery period. ANOVA did not demonstrate the significant effect of POM supplementation on any of these parameters. None of the study groups showed significant changes in ferritin levels at any point of the study (Fig. 3c). The results for sTfR are presented in Fig. 3d. A significant post-exercise increase in sTfR was observed at baseline in the supplemented group ($p < 0.04$), along with a significant decrease in this parameter in the controls. Post-intervention, athletes from the supplemented group presented with significantly higher pre-exercise levels of sTfR than the controls ($p < 0.012$). Pre- and post-supplementation changes in IL-6 levels are shown in Fig. 4. As demonstrated in the ANOVA, physical exercise had a significant effect on the concentration of this cytokine ($p < 0.02$). Prior to the supplementation period, athletes from both groups showed a significant post-exercise increase in IL-6 levels, which persisted post-recovery. This effect was no longer observed at the end of the follow-up period.

Discussion

In this study, supplementation with pomegranate fruit juice boosted the antioxidant potential of rowers, as expressed by TAC. The level of this parameter in the supplemented group was significantly higher during the restitution period than in the placebo group (Fig. 1a).

Table 3 Changes in a 2000 m rowing ergometer performance before and after the supplementation

Parameters	Supplemented group ($n = 10$)		Control group ($n = 9$)	
	Before	After	Before	After
Power (watt)	432 ± 38.4	439 ± 36.4	424 ± 41.3	430 ± 45.6
(W/kg)	4.92 ± 0.29	4.93 ± 0.33	4.96 ± 0.24	5.03 ± 0.20
LA_{min} (mmol/L)[a]	1.8 ± 0.48	1.5 ± 0.26	1.8 ± 0.25	1.6 ± 0.23
LA_{max} (mmol/L)[a]	15.2 ± 2.72	16.33 ± 4.11	13.0 ± 1.84	14.44 ± 2.97
Time (s)	373 ± 11.5	371 ± 10.3	375 ± 12.02	378 ± 15.56

Values represent mean ± standard deviation. No significant differences were found between pre- and post-supplementation parameters ($p < 0.05$)
[a]LA lactic acid

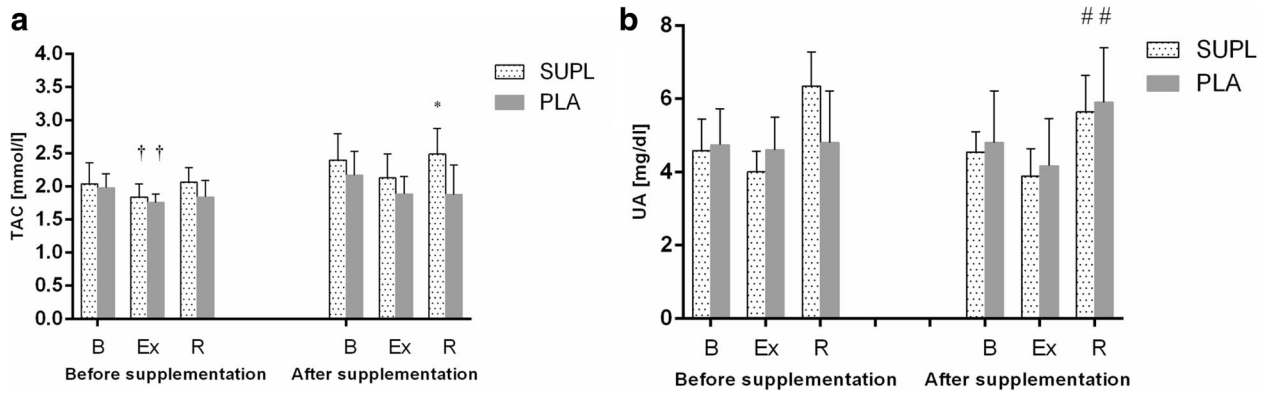

Fig. 1 Data represents the mean (SD) values for total antioxidant capacity (TAC) (**a**) and uric acid (UA) (**b**) levels during exercise tests performed before and after supplementation (mean ± SD). Note: TAC = total antioxidant capacity; UA = uric acid; - SUPL = supplemented group; - PLA = placebo group; B = baseline; Ex = immediately after the exercise; R = after a 1-day recovery; † – significantly different compared to baseline level; * - significantly different compared to PLA; # - significantly different compared to post-exercise level

The increase in antioxidant potential did not exert a significant effect on other study parameters, however. Previous studies [23] have demonstrated that pomegranate fruit juice has three-fold greater antioxidant activity than other food products widely recognised for their antioxidant properties, such as red wine and green tea. The antioxidant potential of pomegranate fruit juice results from its high content of polyphenols, especially proanthocyanidins [24]. An increase in TAC after a two week supplementation with pomegranate fruit juice has also been reported by other authors [25].

Prior to the supplementation (at baseline), intense physical exercise resulted in a significant decrease in TAC in the study athletes (Fig. 1). Free radicals that are accumulated in excess and inadequately inactivated may, inter alia, initiate the peroxidation of polyunsaturated fatty acids of erythrocyte membranes, and thus enhance post-exercise haemolysis [26, 27]. This hypothesis might also be supported by the observation that prior to supplementation, our rowers showed greater post-exercise increases in iron concentration (Fig. 2c). Manthou et al. [28] demonstrated that healthy subjects supplemented

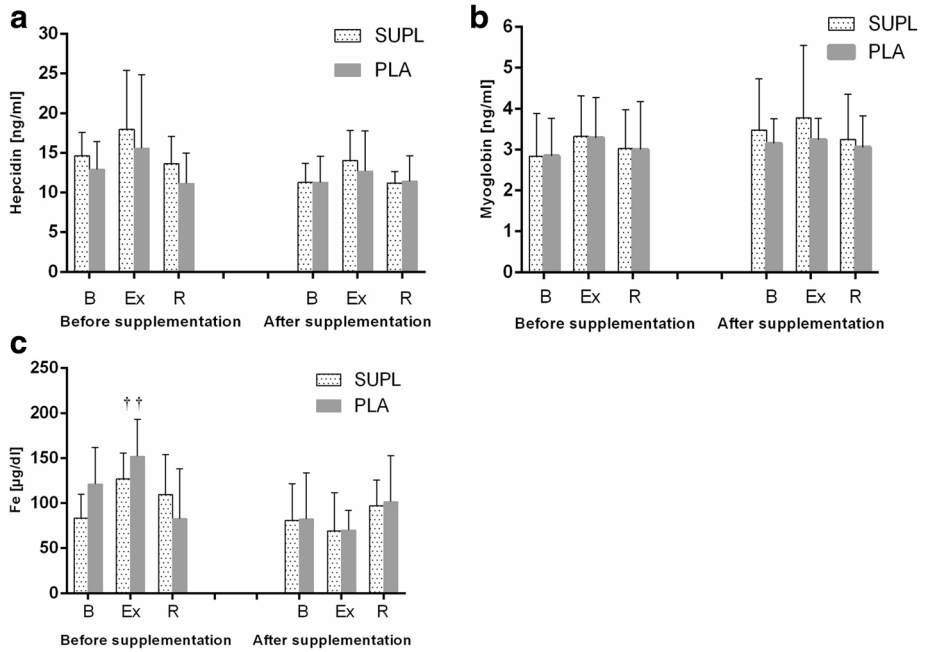

Fig. 2 Data represents the mean (SD) values for hepcidin (**a**), myoglobin (**b**) and iron (Fe) (**c**) levels during exercise tests performed before and after the supplementation (mean ± SD). Note: Fe = iron; - SUPL = supplemented group; - PLA = placebo group; B = baseline; Ex = immediately after the exercise; R = after a 1-day recovery; † – significantly different compared to baseline level

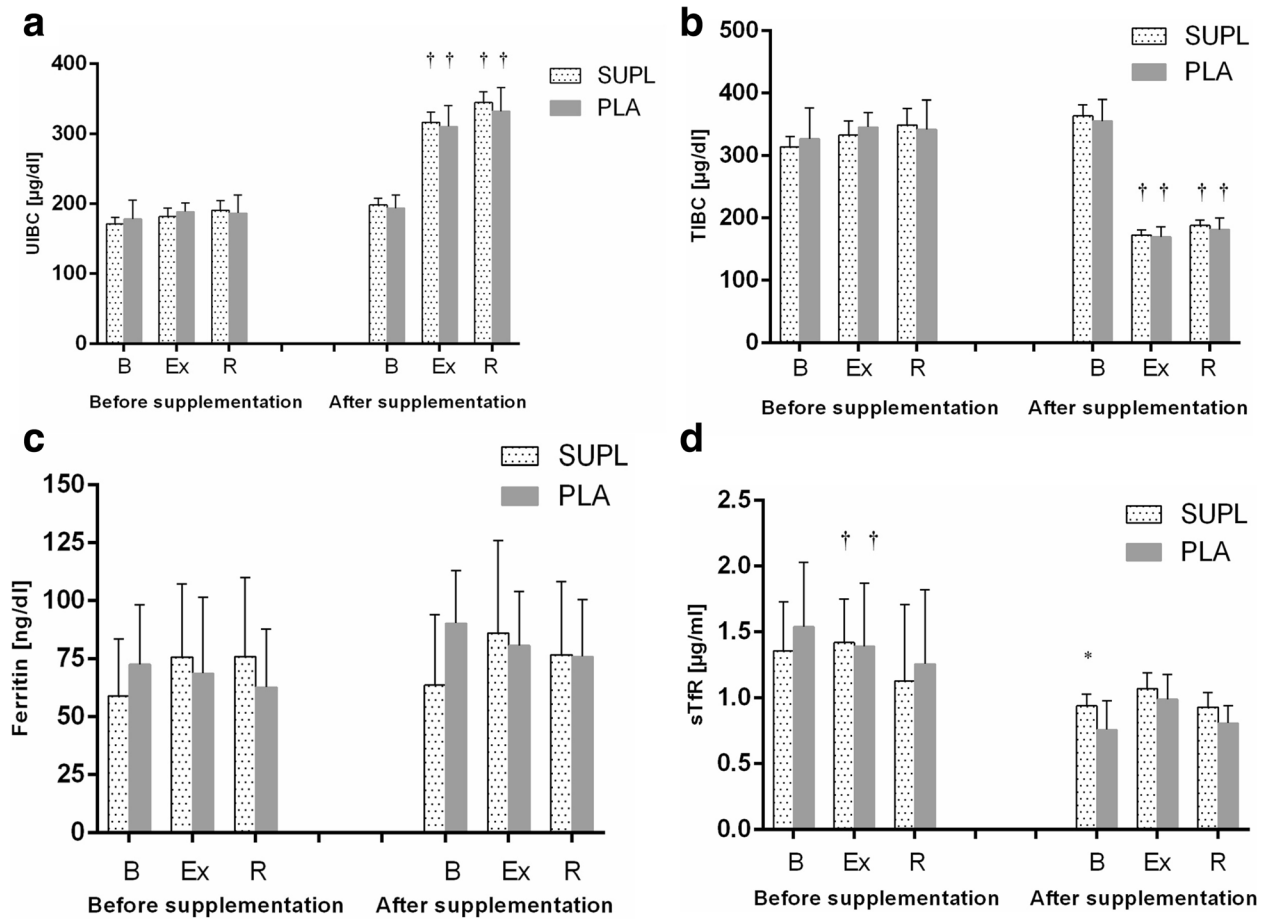

Fig. 3 Data represents the mean (SD) values for iron binding capacity (UIBC) (**a**), total iron binding capacity (TIBC) (**b**), ferritin (**c**) and soluble transferrin receptors (sTfR) (**d**) levels during exercise tests performed before and after the supplementation (mean ± SD). Note: UIBC = unsaturated iron binding capacity; TIBC = total iron binding capacity; sTfR = soluble transferrin receptors; - SUPL = supplemented group; - PLA = placebo group; B = baseline; Ex = immediately after the exercise; R = after a 1-day recovery; † – significantly different compared to baseline level; * - significantly different compared to PLA

for 14 days with pomegranate fruit juice had increased RBC count, haemoglobin concentration and haematocrit levels. According to those authors, these favourable changes might result from more effective prevention of RBC degradation among other thinks. Fiorani et al. [29] demonstrated that human erythrocytes can absorb extracellular flavonoids via passive diffusion, and constitute a reservoir of these compounds. While most flavonoids (according to the authors, up to 85%) reach the cytosol, some are incorporated into cell membrane. Studies [30, 31] have shown that, similar to cholesterol and alpha-tocopherol, intracellular flavonoids are localised in close proximity to the cell membrane, between the lipid bilayer and aqueous phase. As a result of this location, flavonoids play a vital role in the cell, stabilising plasma membranes that become less fluid, and thus, more resistant to oxidation [32]. Another key issue is cooperation between flavonoids, alpha-tocopherol and ascorbic acid. Flavonoids were shown to inhibit the oxidation of intracellular alpha-tocopherol and to regenerate (as does vitamin C) oxidised alpha-tocopherol to its radical. Ascorbic acid, also protected by flavonoids against oxidation, can in turn inhibit oxidative changes in flavonoids, prolonging their protective effect [33, 34]. Flavonoids therefore maintain a relative balance between oxidised and reduced forms of antioxidants and their radicals, and therefore provide another protective mechanism against elevated concentrations of reactive oxygen species.

Although only athletes from the supplemented group presented with enhanced antioxidant potential during the ergometric test conducted at the end of the follow-up period, physical exercise did not induce significant changes in TAC in either study group (Fig. 1a). Uric acid, the final product of purine metabolism, which proved to be an important antioxidant of blood plasma during in vivo studies [35], did not contribute to changes in TAC levels, although our rowers presented with elevated

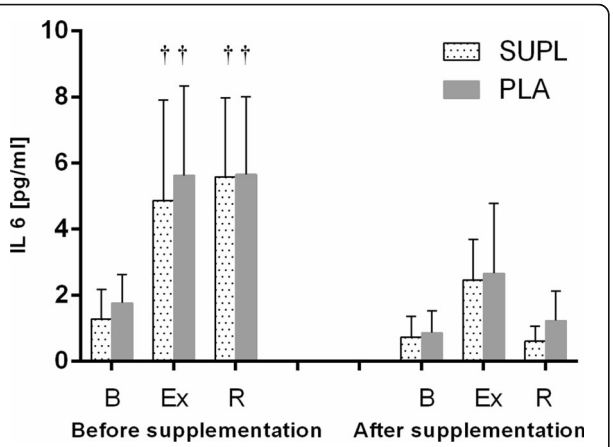

Fig. 4 Data represents the mean (SD) values for interleukin 6 (IL-6) levels during exercise tests performed before and after the supplementation (mean ± SD). Note: IL 6 = interleukin 6; SUPL = supplemented group; PLA = placebo group; B = baseline; Ex = immediately after the exercise; R = after a 1-day recovery; † – significantly different compared to baseline level

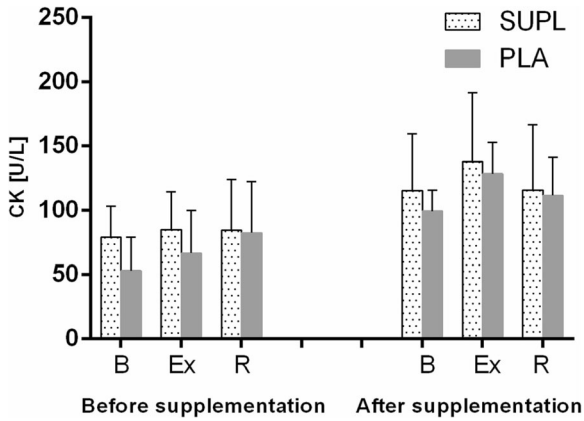

Fig. 5 Data represents the mean (SD) values for creatine kinase (CK) levels during exercise tests performed before and after the supplementation (mean ± SD). Note: CK = creatine kinase; SUPL = supplemented group; PLA = placebo group; B = baseline; Ex = immediately after the exercise; R = after a 1 – day recovery

concentrations during the restitution period (Fig. 1b). Braakhuis et al. [36] demonstrated that the result of a 30 min rowing-ergometer test correlated positively with years and hours of training and the antioxidant status of the blood in elite rowers. According to those authors, these factors had a greater impact on TAC than the dietary intake of antioxidants. The results of our present study suggest that another modulator of TAC may be the phase of the training cycle. The second ergometric test took place during the competitive period when the organism of a well-trained athlete should be characterised by so-called "readiness for competition", that is be fully adapted to an exercise load specific for a given discipline. It should be stressed that during rowing competitions, athletes participate in qualification and final races, and sometimes need to cover a 2000 m distance twice in a single day. The adaptation of our rowers to this type of exercise load was confirmed by other parameters analysed: a lack of statistically significant changes in IL-6 concentration (Fig. 4) and post-exercise increases in iron levels (Fig. 2c). A study of elite male rowers conducted prior to the Rowing World Championships showed a significant association between the level of proinflammatory cytokines, such as IL-1β, TNF-α and IL-6, and measures of depressed mood, sleep disturbances and fatigue [37]. The lack of statistically significant post-exercise changes in concentrations of pro-inflammatory cytokines may thus provide important information about the readiness of athletes for competition.

Irrespective of the testing period, our athletes did not show statistically significant changes in hepcidin, myoglobin or CK levels (Fig. 2a, b, Fig. 5). To the best of our knowledge, CK activity has rarely been studied in POM-supplemented subjects. We found only one report documenting a significant increase in CK activity in a group of recreationally active males receiving either POM or a placebo for a period of nine days; this effect was probably a consequence of myocyte damage in both study groups [38]. The lack of significant changes in hepcidin, myoglobin and CK levels in our study subjects could perhaps be explained by their good adaptation to large training loads; this issue seems to be an interesting topic for future research. Nevertheless, athletes from both groups showed a significant post-exercise increase in serum concentration iron at baseline measurements (Fig. 2c).

Both supplemented athletes and controls showed a significant post-exercise increase in UIBC during the follow-up test, which persisted after a 24 h recovery (Fig. 3a). Similarly, the post-exercise changes in TIBC seemed to be supplementation-independent, since a significant post-exercise decrease in this parameter was observed post-intervention regardless of the study group, both immediately after the ergometric test and following a 24 h recovery (Fig. 3b). Monitoring of sTfR and body iron has previously shown to be a reliable tool for the determination of Fe metabolism and successful prevention of its deficiency [39]. In our present study, ergometric tests conducted at the baseline contributed to a significant increase in sTfR level in the supplemented group and to a significant decrease in this parameter in the controls. Noticeably, athletes from the supplemented group presented with significantly higher pre-exercise levels of sTfR than the controls during the post-intervention ergometric test (Fig. 3d). Neither supplementation with POM nor physical exercise had a significant effect on serum ferritin levels in our study

subjects (Fig. 3c), which is consistent with the results of other studies [40, 41].

Conclusions

This study showed that the administration of pomegranate fruit juice, a dietary supplement with established high antioxidant potential, boosted the TAC of the study athletes, but had no significant effect on inflammatory markers or other parameters analysed. In the case of well-trained athletes, the training phase and adaptation to exercise loads also seem to be important determinants, but this hypothesis needs to be verified by further comprehensive studies.

Abbreviations

ANOVA: Analysis of variance; Fe: Serum iron; IL - 1β: Interleukin 1 beta; IL-6: Interleukin; LA: Lactic acid; LIP: Labile iron pool; POM: Pomegranate juice; SD: Standard deviations; sTfR: Soluble transferrin receptor; TAC: Total antioxidant capacity; TIBC: Total iron binding capacity; TNF-α: Tumour necrosis factor alpha; UIBC: Unsaturated iron-binding capacity

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author for a reasonable request.

Author contributions

ASS, AU and KA designed the study; PB, AW, KWJ and KA collected the data, AU, EL and ASS interpreted the results and drafted the manuscript. All authors approved the final version of the paper.

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Ethics approval and consent to participate

Experimental procedures and potential risks were discussed with the participants, and informed consent forms were provided and signed prior to inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki, and its protocol was approved by the Local Ethics Committee at Poznan University of Medical Sciences (Decision no. 769/13 of 10 October 2013).

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest, financial or otherwise. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

Publisher's Note

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References

1. Buratti P, Gammella E, Rybinska I, Cairo G, Recalcati S. Recent advances in iron metabolism: relevance for health, exercise, and performance. Med Sci Sports Exerc. 2015;47(8):1596–604.
2. Otto J, Montgomery H, Richards T. Haemoglobin concentration and mass as determinants of exercise performance and of surgical outcome. Extrem Physiol Med. 2013;2(1):33.
3. Gozzelino R, Arosio P. Iron homeostasis in health and disease. Int J Mol Sci. 2016;17(1):1–14.
4. Dahlquist DT, Stellingwerff T, Dieter BP, McKenzie DC, Koehle MS. Effects of macro- and micronutrients on exercise-induced hepcidin response in highly trained endurance athletes. Appl Physiol Nutr Metab. 2017;42(10):1036–43.
5. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004; 306(5704):2090–2093.
6. Kong W, Gao G, Chang Y. Hepcidin and sports anemia. Cell Biosc 2014;419. https://doi.org/10.1186/2045-3701-4-19.
7. Sukumaran A, Varghese J, Tamilselvan J, Jeyaseelan V, Mani T, Simpson RJ, et al. Effects of acute and chronic inflammation on proteins involved in duodenal iron absorption in mice: a time-course study. Bri Journal Nutri 2012;108(11):1994–2001.
8. Yu-Qian L, Yan-Zhong C, Bin Z, Hai-Tao W, Xiang-Lin D. Does hepatic hepcidin play an important role in exercise-associated anemia in rats? Int J Sport Nutr Exerc Metabol. 2011;21(1):19–26.
9. Reinke S, Taylor WR, Duda GN, von Haehling S, Reinke P, Volk H. et al. Absolute and functional iron deficiency in professional athletes during training and recovery. Internat J Cardiol 2012; 156(2):186–191.
10. Merkel D, Huerta M, Grotto I, Blum D, Tal O, Rachmilewitz E. et al. Prevalence of iron deficiency and anemia among strenuously trained adolescents. J Adolesc Health 2005;37(3):220–223.
11. Clénin GE, Cordes M, Huber A, Schumacher YO, Noack P, Scales J. et al. Iron deficiency in sports - definition, influence on performance and therapy Swiss Med Wkly 2016;64(1):6–18.
12. Ishibashi A, Maeda N, Kamei A, Goto K. Iron supplementation during three consecutive days of endurance training augmented hepcidin levels. Nutrients. 2017;9(8). https://doi.org/10.3390/nu9080820.
13. Reardon TF, Allen DG. Iron injections in mice increase skeletal muscle iron content, induce oxidative stress and reduce exercise performance. Exp Physiol. 2009;94(6):720–30.
14. Berzosa C, Gómez-Trullén EM, Piedrafita E, Cebrián I, Martínez-Ballarín E, Miana-Mena FJ, et al. Erythrocyte membrane fluidity and indices of plasmatic oxidative damage after acute physical exercise in humans. Eur J Appl Physiol 2011;111(6):1127–1133.
15. Gil M, Tomás-Barberán F, Hess-Pierce B, Holcroft DM, et al. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 2000;48: 4581–4589.
16. Faria A, Monteiro R, Mateus N, Azeveda I, Calhau C. Effect of pomegranate (Punica granatum) juice intake on hepatic oxidative stress. Eur J Nutr. 2007;46:271–8.
17. Shema-Didi L, Sela S, Ore L, Shapiro G, Geron R, Moshe G, Kristal B. One year of pomegranate juice intake decreases oxidative stress, inflammation, and incidence of infections in hemodialysis patients: a randomised placebo controlled trial. Free Radic Biol Med. 2012;53:297–304.
18. Fuster-Mũnoz E, Roche E, Funes L, Martinez-Peinado P, Sempere JM, Vicente-Salar N. Effects of pomegranate juice in circulating parameters, cytokines, and oxidative stress markers in endurance-based athletes: a randomised controlled trial. Nutrition. 2016;32:539–45.
19. Ammar A, Turki M, Chtourou H, Hammouda O, Trabelsi K, Kallel C, et al. Pomegranate supplementation accelerates recovery of muscle damage and soreness and inflammatory markers after a weightlifting training session. PLoS One, 2016:1–19.
20. Căta A, Ştefănuţ MN, Pop R, Tănasie C, Moşoarcă C, Zamfir AD, et al. Evaluation of antioxidant activities of some small fruits containing anthocyanins using electrochemical and chemical methods. Croat Chem Acta 2016; 89(1):37–48.
21. Karlsen A, Retterstøl L, Laake P, Paur I, Kjølsrud-Bøhn S, Sandvik L, et al. Anthocyanins inhibit nuclear factor-kb activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. J Nutr 2007;137(8):1951–1954.

22. Kelsey N, Hulick W, Winter A, Ross E, Linseman D. Neuroprotective effects of anthocyanins on apoptosis induced by mitochondrial oxidative stress. Nutr Neurosc. 2011;14(6):249–59.
23. Gil M, Holcroft D, Kader A, Tomas-Barberan A, Hess-Pierce B. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem. 2000;48(10):4581–9.
24. El Kar C, Ferchichi A, Attia F, Bouajila J. Pomegranate (Punica granatum) juices: chemical composition, micronutrient cations, and antioxidant capacity. J Food Sci. 2011;76(6):C795–800.
25. Naghizadeh-Baghi A, Mazani M, Shadman-Fard A, Nemati A. Punica granatum juice effects on oxidative stress in severe physical activity. Mater Sociomed. 2015;27(1):48–51.
26. Beneke R, Bihn D, Hütler M, Leithäuser R. Haemolysis caused by alterations of α- and β-spectrin after 10 to 35 min of severe exercise. Eur J Appl Physiol. 2005;95(4):307–12.
27. Bonilla JF, Narváez R, Chuaire L. Sports as a cause of oxidative stress and hemolysis. Colombia Medica. 2005;36(4):281–6.
28. Manthou E, Gergakouli K, Deli CK, Sotiropoulos A, Fatouros IG, Kouretas D, et al. Effect of pomegranate juice consumption on biochemical parameters and complete blood count. Exp Therap Med 2017;14:1756–1762.
29. Fiorani M, Accorsi A, Cantoni O. Human red blood cells as a natural flavonoid reservoir. Free Radic Res. 2003;37(12):1331–8.
30. Arora A, Byrem TM, Nair MG, Strasburg GM. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys. 2000;373(1):102–9.
31. Terao J, Piskula M, Yao Q. (1994). Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Arch Biochem Biophys. 1994;308(1):278–84.
32. Cherubini A, Beal MF, Frei B. Black tea increases the resistance of human plasma to lipid peroxidation in vitro, but not ex vivo. Free Radic Biol Med. 1999;27(3–4):381–7.
33. Block G, Henson D, Levine M. Vitamin c: a new look. Ann Intern Med. 1991; 114(10):909–12.
34. Clemetson CA, Andersen L. Plant polyphenols as antioxidants for ascorbic acid. Ann N Y Acad Sci. 1966;136(14):341–76.
35. Kaur H, Halliwell B. Action of biologically-relevant oxidizing species upon uric acid. Identification of uric acid oxidation products. Chem Biol Interact. 1990;73(2–3):235–47.
36. Braakhuis AJ, Hopkins WG, Lowe TE. Effect of dietary antioxidants, training, and performance correlates on antioxidant status in competitive rowers. Int J Sports Physiol Perform. 2013;8:565–72.
37. Main LC, Dawson B, Heel K, Grove JR, Landers GJ, Goodman C. Relationship between inflammatory cytokines and self-report measures of training overload. Res Sport Med. 2010;18(2):127–39.
38. Trombold JR, Reinfeld AS, Casler JR, Coyle EF. The effect of pomegranate juice supplementation on strength and soreness after eccentric exercise. J Strength Cond Res. 2011;25(7):1782–8.
39. Milic R, Martinovic J, Dopsaj M, Dopsaj V. Haematological and iron-related parameters in male and female athletes according to different metabolic energy demands. Eur J Appl Physiol. 2011;111:449–58.
40. Badenhorst C, Dawson B, Cox G, Laarakkers C, Swinkels D, Peeling P, et al. Acute dietary carbohydrate manipulation and the subsequent inflammatory and hepcidin responses to exercise. Eur J Appl Physiol 2015; 115(12), 2521–2530.
41. Burden RJ, Pollock N, Whyte GP, Richards T, Moore B, Busbridge M, et al. Effect of intravenous iron on aerobic capacity and iron metabolism in elite athletes. Med Sci Sports Exerc 2015; 47(7), 1399–1407.