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“Unlocking Athletic Potential: Exploring Exercise Physiology from Mechanisms to Performance”. Acute antioxidant supplementation and performance – should this be considered

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"Unlocking Athletic Potential: Exploring Exercise Physiology from Mechanisms to Performance".

Acute antioxidant supplementation and performance – should this be considered[1]

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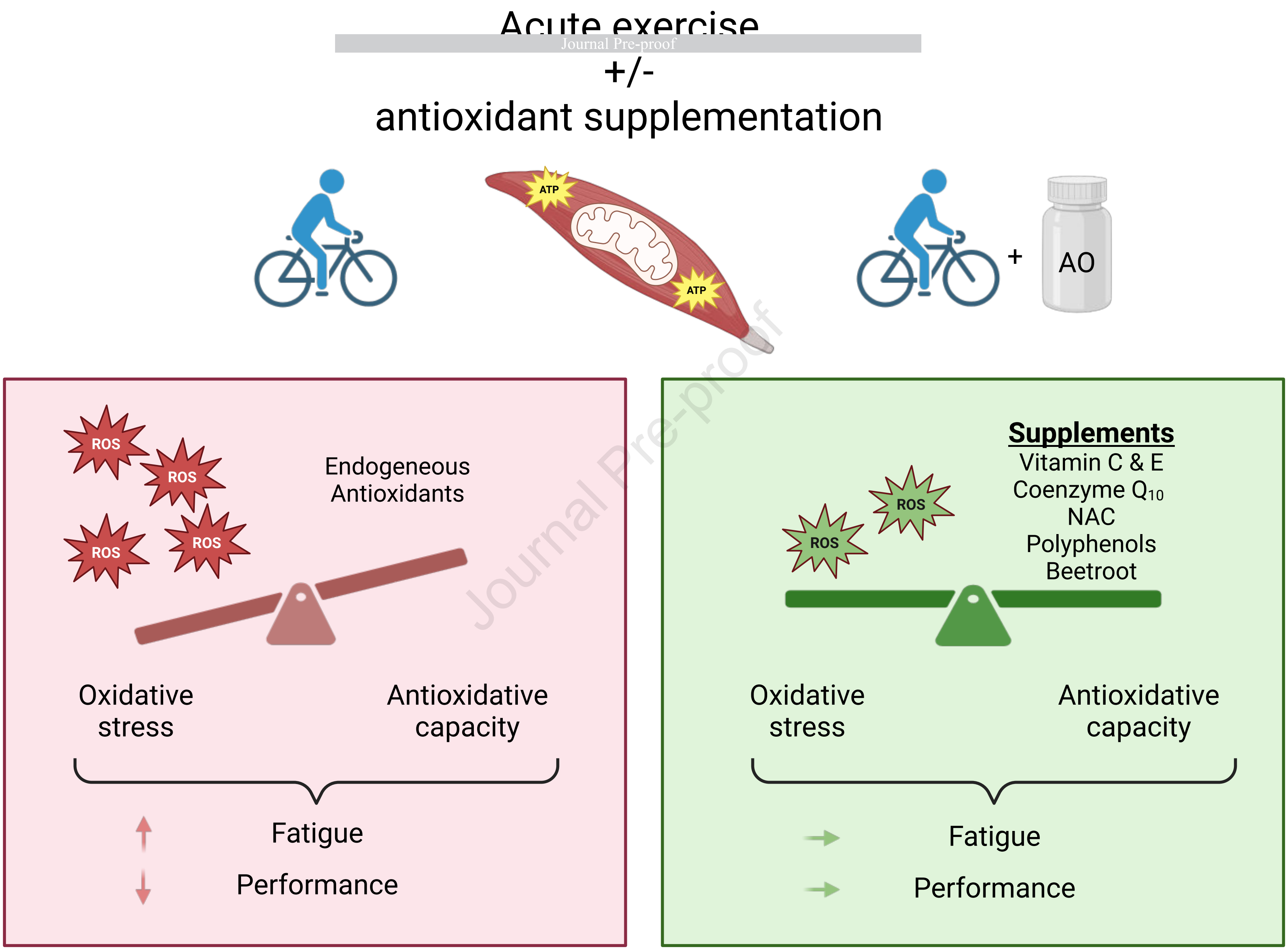
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Performance
Supplements
Vitamin C & E
Coenzyme Q10
NAC
Polyphenols
Beetroot
ROS
ROS
Oxidative
stress
Antioxidative
capacity
Fatigue
Performance

Abstract (max. 200 words)

It is well known that a training intervention leads to mitochondrial adaptations with increased skeletal muscle mitochondrial biogenesis and function. Studies have recently indicated that skeletal muscle mitochondrial function is important for athletic performance. During exercise reactive oxygen species are released from skeletal muscle potentially leading to adaptations but maybe also to fatigue. Focus has been on how chronic antioxidant supplementation affects a training adaptation, where some studies are reporting an abolished adaptation. Whether acute antioxidant supplementation could have a positive effect on fatigue and performance is interesting and highly relevant in sports where athletes are competing over several consecutive days or on the same day, with preliminary competitions in the morning and finals in the afternoon, where it is important for the athletes to recover fast.

This review provides an overview of the effects of acute antioxidant supplementation and whether it leads to improved performance and/or faster recovery in humans.

Introduction

Winning medals at the Olympic Games are settled down to tenths of a second, which is why a lot of time and effort are put into optimization for the athletes. The format in many sports events demands maximal performance more than one time per day, or competing many days in a row at high intensities. This setup makes it very important for the athletes to recover fast. It has been reported that performance decreases (1% in race speed) from qualifying to finals (during the same day) in 4000 meter track cycling[1], which could indicate lack of recovery. Other studies have reported the same drop in performance with multiple maximal efforts over the course of one day[2,3]. These studies imply that intense exercise lasting approximately 4 min can lead to a drop in performance when another bout of intense exercise is performed the same day a few hours later. It needs to be mentioned that high intensity exercise bouts lasting more than 1 min are highly dependent on aerobic energy metabolism, where the mitochondria are important.

Development of muscle fatigue during intense exercise is a very complex process that at the end leads to a decrease in maximal force or power production[4]. Disturbances in different ions (hydrogen, potassium and calcium) in the contractile filaments are linked to muscle fatigue[5]. During high intensity aerobic exercise a transient increase in reactive oxygen species (ROS) production from the skeletal muscle is seen[6], which is beneficial for a training adaptation[7], but potentially negative for performance. The transient increase in ROS might affect potassium handling and calcium sensitivity negatively leading to decreased performance[8]. Studies have shown that mitochondrial oxidative phosphorylation capacity is important for aerobic energy metabolism during intense exercise[9]. Interestingly it has been reported that incubating isolated skeletal muscle mitochondria with H_2O_2 leads to a reduced state 3 respiration and increased state 4 respiration, indicating that the mitochondria are less efficient, which is supported by a reduced P/O ratio[10]. These *ex-vivo* data indicates that the capacity to produce ATP is diminished if ROS production is high during intense exercise, and the efficiency to produce ATP is reduced (lower P/O ratio), leading to a lower total energy production.

During competition it could make sense to try to reduce ROS production in order to improve performance and recovery. The antioxidative system will scavenge ROS production[11], and is increased after a training period[11], but whether acute supplementation with antioxidants will be beneficial in regard to performance and recovery is not that well investigated.

The present review will focus on how acute antioxidant supplementation will influence performance and / or recovery in athletes, competing more than one time per day or consecutive days. Focus will primarily be on oral antioxidant supplementation.

Methods

A literature search was conducted in the database PubMed, with a focus on acute antioxidant supplementation, performance and recovery.

Exercise and mitochondrial function (reactive oxygen species production)

It is well established that training interventions improves mitochondrial function, this is seen with an improved mitochondrial respiratory capacity, content[9] and antioxidative capacity[12]. Hans Hoppeler looked at mitochondrial volume density using electron microscopy before and after 6 weeks of endurance training and found an increased mitochondrial volume density[13]. Different training modalities improves mitochondrial function. It has been reported that as little as 14 days of high intensity training improves mitochondrial respiratory capacity and mitochondrial content, with no changes in intrinsic mitochondrial respiratory capacity[14]. Endurance training for 6 weeks improves mitochondrial content as well as respiratory capacity, but with a reduced intrinsic mitochondrial respiratory capacity[15]. This reduced intrinsic mitochondrial respiratory capacity indicates that mitochondrial volume is increased more than mitochondrial respiratory capacity, which might reduce production of ROS during exercise.

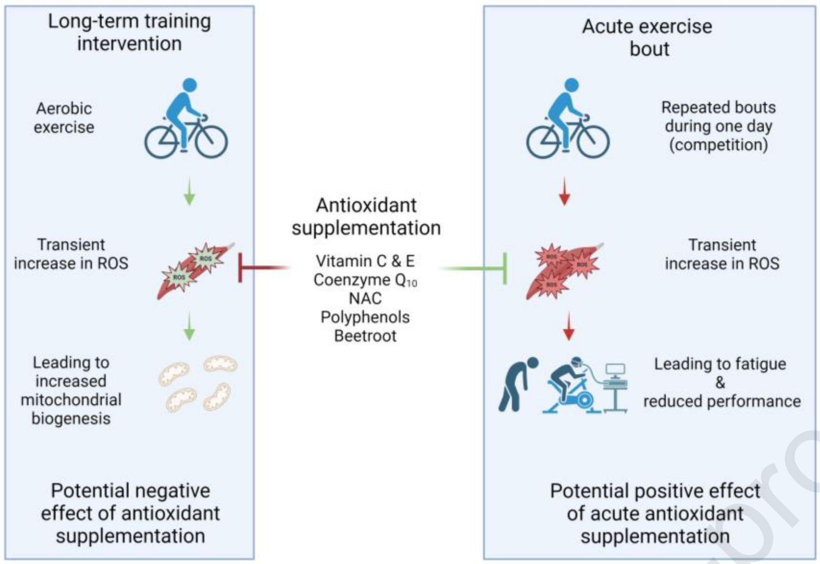

Figure 1: The left side of the figure shows effects of a long-term training intervention, and how antioxidant supplementation could abolish a training adaptation. The right side shows the speculated effect of acute antioxidant supplementation on performance and fatigue. In both situations the increased ROS production after one bout of exercise is important.

Davies and colleagues reported that ROS production increased during exercise and provided a stimulus for mitochondrial biogenesis[16]. Mito-hormesis describes the relationship of low exposure to a stressor will lead to beneficial adaptations[17], during acute exercise ROS will transient increase and will lead to a training adaptation (mitochondrial biogenesis)[7]. The early study by Davies reported increased ROS production following exercise, but the analysis was done on whole muscle tissue[16], and it was therefore not possible to distinguish between mitochondrial or cytosolic derived ROS. Henriquez-Olguin published a review in 2020 highlighting the importance of being able to distinguish between cytosolic and mitochondrial derived ROS production in relation to adaptation[6]. In this review they report that cytosolic ROS is increased during exercise whereas mitochondrial ROS is increased post exercise[6]. These studies indicate that production of ROS during and after exercise is an important signaling molecule for training adaptations (see figure 1). Some studies have shown that removing this signal (ROS) during a training intervention with antioxidant supplementation (chronic exposure to training and antioxidant supplementation) will lead to abolished adaptations[18-20]. It seems as if previous training status could have an influence on the adaptation after antioxidant supplementation[19], unfortunately VO_{2peak} was not reported in the study, which would have been interesting.

Reactive oxygen species and athletic performance

As highlighted in the previous section ROS production from the skeletal muscle (cytosolic and mitochondrial derived) seems to be important in order to induce a training adaptation, and removing this signal with antioxidant supplementation leads to abolished adaptations. Focusing on optimizing performance and reducing fatigue during competition it might be relevant to scavenge ROS with acute antioxidant supplementation.

Accumulation of ROS has been linked to the development of fatigue[21-23]. During exercise oxygen uptake increases in an intensity dependent manner, increasing flux through the electron transport system, leads to an intensity dependent increased ROS production from skeletal muscle[16,24,25]. At higher intensities the endogenous antioxidant defense system are not able to scavenge the ROS produced thereby leading to fatigue and potentially reduced performance (see figure 1). Different mechanisms has been speculated to be responsible for the development of fatigue with high ROS accumulation and impaired calcium handling being some of them. Therefore is seems reasonable to try to manipulate ROS production acutely with antioxidant supplementation in order to improve performance and reduce fatigue.

Antioxidants supplements

In table 1 the antioxidants mostly used as supplements to improve performance are listed with their function briefly described.

Antioxidant	Function
Beetroot juice (nitric oxide)	It is suggested that it leads to increased blood flow and efficiency in the working muscle[26]
Coenzyme Q_{10}	Important for the transport of electrons in the electron transport system in the mitochondria – leading to energy production and an important antioxidant[27]
N-acetyl cysteine (NAC)	Important antioxidant leading to increased concentration of glutathione[28]
Vitamin C	Protects cells from oxidative damage. Potential improvement in aerobe capacity leading to improved performance[29]
Vitamin E	Protects cells from oxidative damage. Potential improvement in aerobe capacity leading to improved performance[29]
Polyphenols	Antioxidative properties potentially leading to mitochondrial biogenesis[30,31]

Acute antioxidant supplementation and exercise performance

In the following section data from studies using acute oral antioxidant supplementation will be covered with focus on performance and recovery, but studies using intravenous infusion will also briefly be mentioned. Acute supplementation is considered to be less than or equal to 7 days. The antioxidants mentioned in Table 1 will be discussed.

Beetroot juice (nitric oxide)

Table 2: Studies investigating the effects of beetroot juice supplementation on performance and recovery.

Reference	Supplement	Participants	Exercise/test	Impact on performance/recovery
Esen et al.[32]	12.8 mmol NO^-_3 supplemented 3 h pre exercise	Trained rugby players (n=12) Crossover design	The Prone Yo-Yo IR1 test and countermovement jumps (CMJ)	No differences were seen in either Yo-Yo test or CMJ
Behrens Jr et al.[33]	6.4 mmol NO^-_3 supplemented 2.5 h pre exercise	Males and females with obesity (n=16; 11 males and 5 females) Crossover design	Time to exhaustion test at 90% of maximal workload	An increased TTE was seen after beetroot juice supplementation, this was accompanied by a reduced oxygen consumption at the given intensity.
Lopéz-Samanes et al.[34]	6.4 mmol NO^-_3 supplemented 3 h pre exercise	Elite female hockey players (n=11) Crossover design	CMJ & isometric handgrip strength & 20 m sprint and repeated sprint ability test. Simulated hockey match play	No differences were seen in neuromuscular performance or match-play demands.
Gleen et al.[35]	8 g of citrulline-malate (CM) (precursor to nitric oxide) 1 h pre exercise	Female master athletes tennis players (n=17) Crossover design	Grip strength, vertical power, Wingate test (anaerobic cycling performance)	Grip strength increased after CM supplementation. Vertical power did not change. Peak power and average power during the Wingate test increased with CM supplementation.
Sandbakk et al.[36]	614 mg nitrate 2.5 h pre exercise	Male elite cross-country skiers (n=9) (VO_{2peak}: 69.3±5.8 ml/min/kg) Crossover design	Running economy (at 10 and 14 km/h with 1% incline. 180 m and 5 km time trial running performance.	No differences were seen in running economy or time trial (180 m or 5 km) after acute supplementation.
Gills et al.[37]	8 g of citrulline-malate (CM) (precursor to nitric oxide) 1 h pre exercise	Recreational active males (n=28) Crossover design	Aerobic cycling test (time to exhaustion (TTE)) and a 30 sec Wingate test	No difference was seen in TTE or total work completed during the aerobic test or mean and peak power during the Wingate test.
MacLeod et al.[38]	6 mmol NO^-_3 supplemented 2 h pre exercise	Trained male cyclist (VO_{2peak} > 60ml/min/kg) At normoxia and moderate hypoxia (2500 m)	15 min steady state exercise at 50% of maximum power output and 10 km time trial	No difference was seen in either steady state exercise or time trial after beetroot juice supplementation. Mean power output during the time trial was higher in normoxia compared to hypoxia.
Wickham et al.[39]	12.8 mmol NO^-_3 supplemented 2 h pre exercise	Recreational active females (n=12)	10 min at 50% of VO_{2peak} and 10 min at 70% of VO_{2peak} followed by a time trial (4 kJ/kg body mass)	No difference was found in oxygen consumption during the two different exercise intensities or time trial performance.
Clifford et al.[40]	A low and high supplementation with beetroot juice. Concentration is not given.	Recreational active males (n=30) allocated to either low or high beetroot juice or placebo (10 in each group=	Muscle damaging exercise bout (100-drop jumps). Counter movement jump (CMJ) height was measured post the muscle damaging exercise and again 24, 48 and 72 h after.	CMJ height was rescued faster with beetroot juice supplementation compared with placebo. Pressure pain threshold was also improved in the two supplemented groups.
Clifford et al.[41]	Beetroot juice supplementation for 3 days	Team-sports players (n=20)	20 maximal-effort 30 m sprints. Counter movement jump (CMJ) height was measured during supplementation an 24 hours after last sprint test.	No difference was seen in sprint performance between the two groups. CMJ height was rescued faster with supplementation.
Jiaqi et al.[42]	6.45 or 12.9 mmol NO^-_3 supplemented	Recreational active women (n=13)	8 x 1 min bouts of cycling at 85% of peak power output with 1 min active recovery. Heart rate (HR), blood	HR at work and in the recovery period was improved with both supplementations and with no difference between the two

	2.5 h pre exercise		lactate and rating of perceived exertion (RPE) were assessed.	concentrations. RPE was also improved with both supplementations and with no difference between the two concentrations.
Moreno-Heredero et al.[43]	6.4 mmol NO_3^- supplemented 3 h pre exercise	Competitive swimmers (n=18; 9 females and 9 males) Crossover design	2 x (6 x 100 m) maximal effort with 40 sec rest between repetitions and 3 min between sets	100 m times showed no differences between groups. Although the supplemented groups decreased the 6th repetition of set 2 compared to the same repetition in set 1.
Esen et al.[44]	12.8 mmol NO_3^- supplemented 3 h pre exercise	Male gymnasts at an international level (n=10) Crossover design	Maximal strength of the upper-leg and upper-arm & muscular endurance	No differences were seen in strength or endurance.
Bailey et al.[45]	5.1 mmol NO_3^- supplemented for 6 days	Recreational active males (n=7) Crossover design	4 min bouts of exercise at low and high exercise intensity was performed.	A reduced oxygen consumption was seen after supplementation at low and high exercise intensity. Indicating an improved efficiency.
Bailey et al.[46]	6 g of l-arginine was ingested 1 h pre exercise	Recreational active males (n=9) Crossover design	6 min bouts of exercise at low and high exercise intensity was performed.	A reduced oxygen consumption was seen after supplementation at low and high exercise intensity. Indicating an improved efficiency.
Vanhatalo et al.[47]	5.2 mmol NO_3^- was ingested 2.5 h pre exercise and supplemented for 5 days more	Recreational active participants (n=8; 3 females and 5 males) Crossover design	Two 5 min exercise bouts at 90% of gas exchange threshold (GET) was done followed by an incremental ramp test. 7 min rest in between and additionally 3 min baseline at 20W or unloaded was done (total of 10 min rest)	A reduced oxygen consumption was seen after 2.5 h and 5 days ingestion / supplementation at moderate intensity (90% GET). During the incremental test, no differences were seen in oxygen consumption.

Beetroot juice containing nitrate or precursors to nitric oxide has been tested acutely in many different participants ranging from elite athletes to recreational active participants at different ages.

Behrens Jr. et al. investigated the effect of nitrate supplementation 2.5 h before exercise composing of at time to exhaustion test at 90% of maximal workload in males and females with obesity. An increased time to exhaustion was found accompanied by a reduced oxygen consumption at the given intensity[33]. Investigating recreational active participants some studies are reporting a beneficial effect of nitrate supplementation with a reduced oxygen consumption at a given exercise intensity[45-47]. In another study in recreational active females heart rate at work and during recovery was lowered with supplementation, indicating a beneficial effect of nitrate[42]. Clifford and colleagues investigated the effect of recovery after muscle damaging exercise in recreational active males, and found that countermovement jumps and pressure pain threshold was improved with supplementation[40]. Additional two studies investigated the effect of nitrate supplementation in recreational active participants on an aerobic time trial[37,39] or a Wingate test[37], where no differences were found in any of the measured outcomes.

When looking at the acute effect of nitrate supplementation in more trained participants the picture is a little more blurry.

Moreno-Heredero and colleagues investigated the effect of nitrate on swimming performance on 2 sets of 6 repetitions and found no overall effect of the supplementation although some of the repetitions were improved after supplementation[43]. In a study in trained male gymnasts no effect were seen after nitrate supplementation on maximal strength or muscular endurance[44]. Clifford and colleagues investigated the effect of 3 days of nitrate supplementation in team-sports players, and found no differences in maximal sprint effort (20 times 30 m sprint), countermovement jump height (used as a measure for recovery) was measured as well and was improved in the supplemented group[41]. Acute nitrate supplementation has also been investigated in trained cyclist and elite cross-country skiers, where focus was on steady state exercise and time trial performance, where no effect was seen on either of the outcomes after acute nitrate supplementation[36,38]. Isometric handgrip strength, sprint abilities and countermovement jump was measured in elite female hockey players, no beneficial effect was seen after ingestion[34]. A Yo-Yo test and a countermovement jump test was performed in trained rugby players, and no changes were seen in the outcomes after ingestion of nitrate[32]. A precursor to nitric oxide (citrulline-malate) was given to female master athletes tennis players, and grip strength, vertical power and a wingate test were performed. Grip strength and the wingate test improved after ingestion of the precursor, with no changes in vertical power[35].

Beetroot juice (nitrate) seems to have an effect in recreational active participants and participants with obesity when it comes to time to exhaustion or oxygen consumption at a given exercise intensity, although not all studies are supporting this. In more trained athletes the results are not that positive, where no effects are seen when it comes to sprint performance, steady state exercise or time trial performance. Interestingly it seems to be beneficial for older athletes when it comes to ingestion of nitrate. Overall there is a huge difference in response to ingestion/supplementation of beetroot juice depending on training status, body composition and age.

Coenzyme Q_{10}

Few studies have been conducted with acute Coenzyme Q_{10} (CoQ_{10}) supplementation. Reviews on long-term supplementation is available, but is not the scope of this review[48,49].

Table 3: Studies investigating the effects of Coenzyme Q_{10} supplementation on performance and recovery.

Reference	Supplement	Participants	Exercise/test	Impact on performance/recovery/stress markers

Diaz-Castro et al.[50]	3 days: Ubiquinone, 120 mg / day, oral	Highly trained males (n=20)	50 km run	Stress markers was not increased to the same extent in the Q_{10} group
Zheng et al.[51]	1 day: Ubiquinone, 30 mg, oral	Non active males (n=11)	10 min on a stationary bike at 30% of HRR	Increased fat oxidation during exercise
Cooke et al.[52]	1 day: 200 mg fastmelt CoQ_{10} formulation	Aerobic trained (n=22) & untrained (n=19) males and females	50 repetition isokinetic leg extension test & 30 sec wingate test & maximal cardiopulmonary test	No differences were observed in the 3 different performance test after acute supplementation with Q_{10}. No difference was seen in stress markers between the two groups in 8-Isoprostane, but an increase over time during the different test performed.

Abbreviations: HRR: Heart Rate Reserve

Highly trained male participants were investigated in a cross-sectional design before and after 50 km run, where half was supplemented with CoQ_{10} 3 days pre race[50]. Due to the design of the study no performance parameters were recorded, but different stress markers were measured (hydroperoxides, isoprostanes, 8-OHdG), these markers were all increased in both groups, but to a lesser extent in the CoQ_{10} supplemented group.

Administration of 30 mg CoQ_{10} before 10 min of low intensity exercise in non-active males increased fat oxidation during exercise[51]. In a study by Cooke and colleagues, one administration of CoQ_{10} or placebo was given to a mixture of trained and untrained males and females participants[52]. They performed three different test; Isokinetic leg extension (muscle endurance), Wingate test (anaerobic power) and a maximal cardiopulmonary test (aerobic power) and looked into different markers for oxidative stress. There were no effect of CoQ_{10} supplementation on the different performance test or markers for oxidative stress[52]. The Authors are not looking into the effect of training status and gender in their primary outcomes, which would have been very interesting, they are stating that there are some trends in relation to muscle CoQ_{10} content, where male participants and trained participants seems to have higher levels of muscle CoQ_{10} content.

Few studies have investigated the acute effect of CoQ_{10} supplementation on performance, recovery and oxidative stress, and it seems as if there are no effect on performance after acute supplementation.

Table 4: Studies investigating the effects of NAC supplementation on performance and recovery.

Reference	Supplement	Participants	Exercise/test	Impact on performance/recovery
Christensen et al.[53]	NAC (20mg/kg body weight) was ingested 1 h pre exercise	Well-trained cyclist (VO_{2peak}: 69±7 ml/min/kg) (n=11) Crossover design	Two performance test (4 min)was done on the same day with 90 min rest in between	Mean power and peak oxygen consumption during the tests was not different between the groups. Participant's readiness to the performance test was not different between the groups, but was reduced in both groups at the last test on the same day.
Smith et al.[54]	NAC (70mg/kg body weight) was ingested 1 h pre exercise	Healthy non-endurance trained males (n=8)	Handgrip strength with bloodflow and oxygenation	No differences were found in any of the measured outcomes between the two groups.
Rhodes et al.[55]	NAC (1 g/day) for 6 days	Semi-elite male rugby players (n=17; NAC (n=8) and placebo (n=9))	Muscle soreness and sprint performance were measured at baseline and on day 5 and 6 of supplementation.	No clear results, but it seems as if muscle soreness was improved after the test at day 5 but worsened after the test on day 6. Performance was improved from test day 5 to test day 6
Childs et al.[56]	NAC (10mg/kg body weight) combined with vitamin C (12.5 mg/kg body weight for 7 days	Healthy untrained males (n=14; NAC + vitamin C (n=7) and placebo (n=7))	Induced an exercise for muscle damage and followed markers for oxidative stress during the following 7 days.	Markers for oxidative stress and muscle damage increased in the days following the muscle damaging exercise bout in the supplemented group.
Tan et al.[57]	NAC (70mg/kg body weight) was ingested 1 h pre exercise	Recreational active males (VO_{2peak}: 52±8 ml/min/kg) (n=16)	1 hour heavy-intensity cycling followed by a time-to-exhaustion test	No differences were found in exercise performance after NAC supplementation.
Corn et al.[58]	NAC (70mg/kg body weight) was ingested 1 h pre exercise	Recreational active males (n=7) Crossover design	Time to fatigue (at 80, 90, 100 and 110 % of peak power output) and critical power.	NAC improved time to fatigue at 80% of peak power output, but no other differences were observed.
Bailey et al.[59]	Intravenous infusion of NAC (125mg/kg/h for 15 min followed by 25mg/kg/h for	Recreational active males (n=8) (VO_{2peak}: 51±9 ml/min/kg) Crossover design	Endurance exercise test were performed	No differences were seen in the performance test.

	the rest of the exercise protocol			
Medved et al.[60]	Intravenous infusion of NAC (125mg/kg/h for 15 min followed by 25mg/kg/h for the rest of the exercise protocol	Active males (VO_{2peak}: 52.3±2.8 ml/min/kg) (n=8) Crossover design	45 min at 70% of VO_{2peak} followed by exercise at 90% of VO_{2peak} until fatigue	No differences were found in the two performance test, but it seems as those participants with the highest VO_{2peak} had an improved performance after NAC infusion.
Medved et al.[61]	Intravenous infusion of NAC (125mg/kg/h for 15 min followed by 25mg/kg/h for the rest of the exercise protocol	Well-trained males (VO_{2peak}: 65.6±2.2 ml/min/kg) (n=8) Crossover design	45 min at 70% of VO_{2peak} followed by exercise at 90% of VO_{2peak} until fatigue	Time to fatigue increased with app. 25% after NAC infusion in well trained males.

Abbreviations: NAC: *N*-acetyl cycteine; $VO2_{peak}$: Peak maximal oxygen uptake

N-acetyl cysteine (NAC)

Christensen and Bangsbo used a crossover design to test if acute oral supplementation with NAC would have a positive effect on performance using a test lasting 4 minutes[53]. The study was conducted in well-trained cyclist (VO_{2peak}: 69±7 ml/min/kg) using a NAC supplementation of 20 mg/kg bodyweight, the performance test conducted was separated by 90 minutes. No differences were seen in mean power output between trial 1 and 2 and no effect of NAC supplementation was observed. Total antioxidant capacity was also measured in plasma throughout the test day, and no differences were found[53], a limitation to the study was, that NAC was not measured during the intervention.

In a study by Smith and colleagues, NAC supplementation (70 mg/kg bodyweight) was given orally 1 h before exercise (handgrip), where blood flow and oxygenation was measured. NAC and total cysteine was measured after supplementation and was increased in the NAC group compared with the placebo, indicating that the supplementation was taken. Time to exhaustion, blood flow at rest and during exercise and oxygenation were not different between the two conditions[54].

Rhodes and colleagues investigated the effect of six days of oral NAC (1 g) or placebo supplementation on muscle soreness and sprint performance in semi-professional male rugby players[55]. The performance test were place at baseline and on day 5 and 6 of the supplementation, muscle soreness were monitored every day. In general there were no clear results from this study, but it seems as if performance was improved from day 5 to day 6, results on muscle soreness seems to be improved after the test on day 5, but worsened after the test on day 6.

In a study by Childs and colleagues eccentric exercise was used to induce muscle damage, which was followed by combined vitamin C (12.5 mg / kg body weight) and NAC (10 mg / kg body weight) supplementation for 7 days[56]. Lipid Hydroperoxides, 8-isoprostanes, total antioxidant capacity and bleomycin Detectable iron were all increased in the supplemented group 2-4 days after the muscle injury, indicating that supplementation with vitamin C and NAC post-injury increases tissue damage and oxidative stress.

Tan and colleagues investigated the combination of beetroot (12.4 mmol of NO_3^-)) and NAC (70 mg / kg body weight) or only NAC in recreational active males (VO_{2peak}: 52±8 ml/min/kg), in this study beetroot was supplemented for 6 days prior to the intense exercise test and NAC was supplemented 1 hour prior to exercise[57]. No changes were found in exercise performance after the two different supplementation regimes compared with a placebo condition.

Time to fatigue and critical power was measure after acute oral NAC supplementation (70 mg/kg), and increase in glutathione was seen pre exercise in the NAC group[58]. An improved time to fatigue was seen at 80% of P_{max} in the NAC group and critical power was increased as well, indicating that acute NAC supplementation has an effect on performance. In this study no information is given on subjects VO_{2peak}.

Bailey and colleagues used a different approach, where they were infusing NAC during exercise[59]. Infusion of 125 mg/kg/h for 15 minutes followed by 25 mg/kg/h for the rest of the exercise bout. NAC was measured and increased after infusion as expected, but no change was seen in exercise tolerance, the authors highlights the significant inter-subject variability in response to exercise, indicating that NAC supplementation might be beneficial for some athletes, but that it needs to be individualized.

Medved and colleagues[60] also infused NAC (125 mg/kg/h for 15 minutes followed by 25 mg/kg/h for the rest of the exercise bout) to participants (VO_{2peak}: 52.3±2.8 ml/min/kg), the performance protocol was 45 min at 70% of VO_{2peak} followed by 90% of VO_{2peak} to fatigue. No differences were found in time to fatigue at the whole group level, but those participants with the highest VO_{2peak}, showed improved time to fatigue compared with those with a lower VO_{2peak}. These data indicates that there could be a major difference in the response to acute NAC supplementation depending on fitness level. These data was supported by the same authors using the same approach, but where focus was on more trained participants (VO_{2peak}: 65.6±2.2 ml/min/kg), where time to fatigue increased by over 25%[61]. Interestingly NAC was measured in skeletal muscle in this study and was increased at fatigue[61], which could indicate that when NAC is given intravenously it reached the muscle and a positive effect is seen.

It seems as if acute oral NAC supplementation does not have any massive effect on performance or recovery from the studies mentioned in this section. This could be because of the bioavailability of the compound. It has been reported that *N*-Acetyl cysteine ethyl ester (NACET), has a better pharmacokinetic profile compared with NAC[62], which is something to be considered for future studies. Studies that infuses NAC intravenously seems to have a better effect, but this approach is not very practical and there could be issues with approval of intravenous delivery. Furthermore it also seems as if the effects could be related to fitness level but also to the protocol used for the performance test in relation to length and intensity, when NAC is given intravenously and the performance test last longer (>45 min) infusion has a positive effect[61,63].

Vitamin C and E or combined

Table 5: Studies investigating the effects of vitamin C and/or E supplementation on performance and recovery.

Reference	Supplement	Participants	Exercise/test	Impact on performance/recovery
Lee et al.[64]	Vitamin C was ingested 1 h pre exercise	Middle aged triathlon athletes (n=12; males (n=9) and females (n=3)) (VO_{2peak}: 46±7 ml/min/kg)	90 min of exercise at 70% of VO_{2peak}	No differences were seen in the exercise performance test.
Martínez-Ferrán et al.[65]	Vitamin C and E was ingested 2 h pre exercise	Recreational endurance trained male runners (n=18; (n=9 in vitamin and n=9 in placebo))	8 bouts of 1 km running at 75% of maximum heart rate, with a focus on exercise-induced muscle damage (EIMD)	No differences were seen in EIMD between the groups.
Thompson et al.[66]	1 g vitamin C was ingested 2 h pre exercise	Active males (VO_{2peak}: 55±1 ml/min/kg) (n=9) Crossover design	Muscle soreness and isokinetic muscle function was measured.	No effect of vitamin C ingestion was seen.
[67]de Oliveira et al.[67]	Vitamin C (500mg/day) and vitamin E (400UI/d) was supplemented for 7 days.	Football athletes (n=21)	Plyometric jumping and strength resistance sets to exhaustion was measured. Delayed-onset of muscle soreness (DOMS) was measured before and 24, 48 and 72 h after exercise.	Oxidative stress markers was increased the days after exercise in the placebo group, but was not increased in the supplemented group. Vitamin C and E supplementation did not improve DOMS.

Lee and colleagues tested the effect of vitamin C on exercise performance in middle aged trained triathletes (males and females) and found no effect of supplementation on exercise performance[64].Vitamin C and E supplementation has been investigated in relation to delayed-onset of muscle soreness and exercise-induced muscle damage, where no improvements were observed after supplementation[65,66]. Delayes-onset of muscle soreness was investigated after Vitamin C and E supplementation and was not improved, but different oxidative stress markers were increased the days after exercise in the non supplemented group[67].

In summary vitamin C and/or E does not seem to have a positive impact when it comes to performance or recovery after exercise in relation to delayed-onset of muscle soreness.

<u>Polyphenols</u>

Polyphenols (PP) derives from different fruits and possesses antioxidant and anti-inflammatory properties.

Table 6: Studies investigating the effects of polyphenols supplementation on performance and recovery.

Reference	Supplement	Participants	Exercise/test	Impact on performance/recovery
Cases et al.[68]	290 mg PP from green tea, grape and pomegranate ingested 1 h pre exercise	Recreational active males (n=15) Crossover design	4x30 sec Wingate test with 4-5 min recovery	Increased peak power and average power during the Wingate test.
Oh et al.[69]	72 mg PP from Ecklonia cava ingested 30 min pre exercise	Recreational active males (n=20) Crossover design	Maximal incremental treadmill test	Increased time to exhaustion was found in the PP groups as well as a 6.5% non-significant increase in VO_{2peak}.
Deley et al.[70]	500 mg PP from grape and appels (n=24) and placebo (n=24)	Recreational active males (n=48) Randomized controlled trial	Time to exhaustion at 70% of maximal aerobic power	Increased time to exhaustion in the PP group.
Trexler et al.[71]	1000 mg pomegranate 30 min pre exercise	Recreational active males (n=19) Crossover design	Three treadmill runs to exhaustion at 90, 100 and 110% of peak velocity	Increased time to exhaustion at 90 and 100 % of peak velocity after PP ingestion.
Crum et al.[72]	1000 mg pomegranate ingested 2.5 h pre exercise	Highly trained cyclist (n=8) (VO_{2peak}: 74.4±6.2 ml/min/kg)	3 times 6 min (50, 65, 80% of VO_{2peak} followed by a time to	Time to exhaustion was not different between group, but was lower at altitude compared to sea level. It seems as if there was a numeric increase driven by 1 participant. VO_{2peak} was increased in PP at altitude compared with placebo at altitude.

		Crossover design	fatigue at 100% VO_{2peak}. This was done at sea level and 1657 m altitude.	
Decroix et al.[73]	900 mg cocoa flavonols ingested 1.5 h and 3 h pre exercise	Well trained cyclist (n=12) (VO_{2peak}: 63.0±3.5 ml/min/kg) Crossover design	Two cycling time trials (TT) were conducted (30 min at 75% of peak power output) with 100 min rest	Power output during the two time trials was not different although TT1 tended to be improved with cocoa flavonols. Plasma markers for oxidative stress was not different between the groups.
Keane et al.[74]	60 ml Montmorency cherry concentrate ingested 1.5 h pre exercise. Polyphenol dose not given.	Trained cyclist (n=10) (VO_{2peak}: 59.0±7.0 ml/min/kg) Crossover design	2 different setup for each experimental condition. 1: 6 min moderate & 6 min intense cycling continued to exhaustion & 2: followed by an all-out 60 sec sprint	No differences were seen in time to exhaustion, but an increase in peak power and work done during the 60 sec sprint
Labonté et al.[75]	800 mg polyphenols was ingested before exercise, specific time not given	Elite athletes (n=12; 2 females and 10 males) Crossover design	3 km time trial on an ergometer bike	No difference in time trial performance, but heart rate was significantly lower 2 and 5 min post exercise in the PP group.
Parenteau et al.[76]	0.7 g/kg body mass of cranberry powder ingested 1-3 h pre exercise	Endurance athletes (n=14; 6 females and 8 males)	400 and 1500 m time trial running	No differences were seen after acute administration of PP on either 400 or 1500 m time trial.
Roelofs et al.[77]	1000 mg pomegranate ingested 30 min pre exercise	Recreational resistance trained (n=19) Crossover design	Bench and leg press rep. to failure at 80% 1RM and 10x6 sec cycle sprints with 30 sec rest	No differences were seen in bench or leg press repetitions. For the 6 sec sprints no differences were observed although average power was higher in PP in sprint 5 and peak power was higher in sprint 5 and 7, but no main effect of treatment.
Watanabe et al.[78]	500 mg quercetin glycosides with 300 ml water, post measurements were done 1 h after ingestion	Young males (n=13)	Firing rates of motor units recruited at 30-50% of maximal voluntary contraction torque (MVC)	MVC was increased after ingestion of PP.

Abbreviations: PP: Polyphenols

Cases and colleagues supplemented recreational active men 1 h before exercise with 290 mg of PP (green tea, grape and pomegranate), and found increased peak power and average power during four 30 sec Wingate test[68]. Improved time to exhaustion and a 6.5% non-significant increase in VO2peak was seen after ingestion of 72 mg Ecklonia cava 30 min before exercise in recreational active males compared with placebo[69]. Deley and colleagues performed a randomized controlled study measuring time to exhaustion after 500 mg ingestion of PP from grape and apple the evening before and 1 h pre exercise. They found an improved time to exhaustion in the PP group[70]. It would have been interesting to have a baseline measurement since it is a randomized controlled trial and not a crossover design. Trexler et al. investigated the effect of 1000 mg pomegranate 30 min pre exercise in recreationally active males and found improved time to exhaustion at 90 and 100% of peak running velocity[71].

Polyphenols (1000 mg pomegranate given 2.5 h before exercise) was tested in highly trained cyclist at sea level and at altitude (1657 m)[72]. No differences were found in time to exhaustion (TTE) either at sea level or at altitude, but one participant increased TTE dramatically in both conditions, which again highlights the individual response to a supplementation[72]. Maximal oxygen uptake was increased at altitude after PP ingestion with no changes at sea level. In another study in well trained cyclist no effect was seen on time trial performance after acute supplementation although a tendency was observed for a beneficial effect of PP. This was accompanied by no changes in plasma oxidative stress markers[73]. Keane and colleagues investigated the effect of Montmorency cherry concentrate given 1.5 h before exercise and looked at time to exhaustion during an intense cycling exercise as well as a 60 sec sprint performance and found no differences in time to exhaustion but an improved sprint performance with PP (increased peak power and work done)[74]. Running performance were investigated in endurance athletes 1-3 h after ingestion of cranberry powder, no effect was seen on 400 and 1500 m running performance[76].

Strength and anaerobic power was evaluated with or without 1000 mg pomegranate 30 min before exercise in recreational active men. No differences were observed in either numbers of repetitions in bench and leg press at 80% of 1RM or in sprint performance (10 x 6 sec sprints on a bicycle ergometer)[77]. Quercitin (500 mg) was ingested and motor units recruitment was measured at 30-50% of maximal voluntary contraction and was found improved after PP ingestion[78].

It seems as if time to exhaustion is improved with PP ingestion immediately prior to exercise in recreationally active participants, but more trained participant is not getting similar benefits from PP ingestion.

Conclusion

In conclusion, it seems as if the different antioxidants review in this paper, does not have a major effect on performance or recovery, when oral supplemented acutely to highly trained participants. When looking into less trained participants there seems to be an effect of both recovery as well as performance, when using some of the antioxidant mentioned. Furthermore is also seems as if the effects are more beneficial when it comes to middle-aged or older participants. Infusion with NAC in highly trained participants seems to be beneficial in regard to performance.

Fitness level and age seems to be important in relation to the effects of acute oral supplementation with different antioxidants. This discrepancy might be related to the level of oxidative stress at baseline in the participants, but this needs to be investigated further.

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Highlights:

The effects of acute antioxidant supplementation on performance and fatigue in human participants with different cardiorespiratory fitness levels, age and weight was reviewed.

- Beetroot supplementation seems to have a positive effect in recreational active participants, which is not the case in highly trained athletes.
- NAC does not seem to have a beneficial effect on performance and fatigue when given orally.
- Polyphenols seems to have a beneficial effect in recreational active participants, which is not the case in highly trained athletes.
- Fitness level and age seems to be important in relation to the effects of acute oral antioxidant supplementation on performance and fatigue.

No competing interest are related to this review