Effects of Dietary Supplements on Adaptations to Endurance Training

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Effects of Dietary Supplements on Adaptations to Endurance Training

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Abstract
Endurance training leads to a variety of adaptations at the cellular and systemic levels that serve to minimise disruptions in whole-body homeostasis caused by exercise. These adaptations are differentially affected by training volume, training intensity, and training status, as well as by nutritional choices that can enhance or impair the response to training. A variety of supplements have been studied in the context of acute performance enhancement, but the effects of continued supplementation concurrent to endurance training programs are less well characterised. For example, supplements such as sodium bicarbonate and beta-alanine can improve endurance performance and possibly training adaptations during endurance training by affecting buffering capacity and/or allowing an increased training intensity, while antioxidants such as vitamin C and vitamin E may impair training adaptations by blunting cellular signaling but appear to have little effect on
performance outcomes. Additionally, limited data suggest the potential for dietary nitrate (in the form of beetroot juice), creatine, and possibly caffeine, to further enhance endurance training adaptation. Therefore, the objective of this review is to examine the impact of dietary supplements on metabolic and physiological adaptations to endurance training.

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Key Points

- Many supplements have been studied in the context of acute performance enhancement, but the effects of continued supplementation concurrent to endurance training programs are less well characterised.
- Supplements including sodium bicarbonate, beta-alanine, dietary nitrates, antioxidants, caffeine, and creatine have the potential to modify the adaptive response to endurance training (either positively or negatively) by affecting acid-base balance, redox status, oxidant signaling, or cumulative training load, thereby affecting the cellular signaling responses to training.

1. Introduction

Endurance training (repeated sessions of continuous or intermittent exercise performed with the goal of improving endurance performance) leads to metabolic and morphological adaptations at the cellular and systemic levels, which allow submaximal-intensity exercise to be performed with a smaller homeostatic disturbance [1]. These adaptations include increases in maximal oxygen uptake (VO$_{2\text{max}}$), mitochondrial enzyme activity, and mitochondrial protein content, which result in a shift towards greater reliance on fat for fuel, reduced glycolytic flux, decreased lactate accumulation at a given work rate, and tighter control of acid-base balance [1-3].
Training-induced adaptations are the consequence of repeated stimuli from individual exercise sessions [3], and the accumulation over time of transient, exercise-induced changes in gene expression [4]. Manipulation of training intensity and duration are the primary variables affecting the exercise response [5]. However, dietary intake can also impact adaptations to training by increasing the exercise stimulus and/or enhancing or blunting cellular responses to exercise-induced perturbations (Fig. 1) [6].

Elite athletes use dietary supplements more than non-elite athletes, with a similar prevalence between men and women [7]. Although the motivations for using these supplements are often not with the goal of improving adaptations to training, the use of dietary supplements represents an under-researched and under-appreciated approach for impacting the adaptive response to endurance training.

The performance effects of supplements commonly used by endurance athletes, including sodium bicarbonate, β-alanine, and dietary nitrates, has been reviewed [8-13]. However, these supplements are typically considered only in the context of acute performance changes and less is known about how they influence adaptations to training. These supplements have the potential to modify the adaptive response to endurance training (either positively or negatively) by affecting acid-base balance, redox status, reactive oxygen species (ROS) signaling, or cumulative training load, thereby affecting the cellular signaling responses to training (Fig. 2). Some discussion of the effects of supplements on training adaptations has previously been published [14-16], but these were focused on a narrow subset of
supplements and did not include creatine or caffeine, or research published over the past five years. Thus, an updated overview of the current literature is warranted. The objective of this review is to examine the impact of common dietary supplements on the metabolic and physiological adaptations to endurance training.

2. Buffering Agents

Supplements that act on buffering and pH regulation may affect the training response by allowing an athlete to train harder (increasing the exercise stimulus), and/or impacting important signaling molecules, such as the 5' AMP-activated protein kinase (AMPK), p38 mitogen-activated protein kinase (MAPK), and the Ca^{2+}/calmodulin-dependent kinase (CaMK) pathways, which are affected by pH [17-19]. For example, an acidic pH decreased the protein content of phosphorylated AMPK in differentiated L6 myotubes [19], cultured fibroblasts [20], and rat cardiomyocytes [21], while an alkaline pH increased phosphorylated AMPK protein content in rat cardiomyocytes [21]. In humans, inducing acidosis with ammonium chloride before a session of interval exercise blunted the post-exercise increases in peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), citrate synthase (CS), cytochrome C (CYT-C), and GLUT4 mRNA compared with a placebo [22] (Fig. 3). Blood lactate concentrations are also decreased in acidosis, likely due to the attenuation of glycolytic activity [23]. This may negatively impact training adaptations as lactate signaling can influence mitochondrial biogenesis [24] and the chronic adaptive response to training [25]. An increase in blood pH can increase the rate of muscle glycogen breakdown along with increasing blood lactate levels and H^+ efflux out of contracting muscles [26, 27]. Increasing blood pH via ingestion of sodium bicarbonate prior to a session of high-intensity exercise
increased the expression of $PGC-1\alpha$ mRNA to a greater degree than a placebo [27]. Although acute responses to training do not always correspond with long-term adaptations [28], there is evidence to suggest that supplements that alter pH may affect adaptations to training.

2.1 Sodium Bicarbonate

Sodium bicarbonate is typically consumed in a dose of 0.2 to 0.4 grams per kg of bodyweight, divided across one to three servings [16], although serial loading protocols over several days have also been used [29]. This dose should be sufficient to raise bicarbonate concentrations in the blood by 5 to 6 mmol/L and allow small changes in blood pH during sub-maximal exercise, although large interindividual variability in the response to supplementation has been reported [30-32]. Ingestion of sodium bicarbonate before each cycling interval training session (24 sessions over eight weeks) in recreationally-active women resulted in greater improvements in the lactate threshold (26 vs. 15%, $d = 0.5$) and time to fatigue (164 vs 123%, $d = 1.9$) compared with a placebo, even though training volume and intensity was matched between groups (Table 1) [33]. Six weeks of high-intensity interval training (HIIT) (18 sessions) by recreationally-active men consuming sodium bicarbonate or a placebo prior to each training session led to greater improvement in relative peak power (20.8 vs. 10.3%, $d = 2.1$) but no differences in relative mean power on a Wingate test [34], and similar improvements in time to fatigue, maximum power, and lactate threshold power, though only the bicarbonate group had significant increases in CS activity (22.3 vs. 12.6%) [35]. However, four weeks of sodium bicarbonate supplementation in highly-trained male rowers did not provide significant performance benefits compared with a placebo [36]. While favourable trends were observed for 2,000-m power, peak power, and power at the lactate threshold, it
is possible that performing only 8 sessions over the 4-week study period and/or the small sample size (n=6 per group) was not sufficient to detect significant changes.

Rats performing HIIT on a treadmill five times per week for five weeks had a 52% longer time to exhaustion ($d = 11.1$), and greater improvements in ADP-stimulated mitochondrial respiration ($d = 1.8$), when consuming sodium bicarbonate before exercise, compared with rats that ingested a placebo prior to exercise [37, 38]. These groups were also matched for total work performed during each training session, further supporting the potential for bicarbonate to improve adaptations to training. Changes in the activity of CS and phosphofructokinase, and sodium bicarbonate cotransporter protein content, were similar to the placebo group [37, 38], although levels of monocarboxylate transporter 4 increased to a greater degree after training with bicarbonate supplementation [38].

Overall, sodium bicarbonate is unlikely to have an effect on $\text{VO}_{2\text{max}}$ (which is largely affected by central adaptations [39]), but may affect peripheral adaptations that are influenced by pH such as the lactate threshold, CS activity, and mitochondrial respiration, particularly in untrained participants (Fig. 3). Contrasting results may be due to differences in fitness level, number and intensity of training sessions performed, sex differences, genetics, and sample size. Two studies that showed no differences between groups prescribed HIIT at a percentage of peak power [35, 36], while a study that set training intensity as a percentage of the lactate threshold resulted in improved performance [33].
Future research should elucidate differences in mitochondrial enzyme content and respiration as a result of sodium bicarbonate ingestion prior to training sessions. Exercise stimuli should be sufficient to alter lactate levels and/or pH compared with a placebo, and should compare both work-matched and time-based (e.g., 4-min intervals at maximal effort) intervals to examine the effects of differences in pH versus differences in total work during training sessions.

2.2 Beta-alanine

Beta-alanine is an increasingly popular supplement for athletes due to its ability to delay fatigue during high-intensity exercise. It does this by increasing muscle carnosine content in a dose-dependent manner [40], and improvements in exercise performance have been shown with doses ranging from 3.2 to 6.4 g/day for 4 to 24 weeks [9]. β-alanine has the potential to enhance training adaptations by increasing the ability to sustain exercise at a higher intensity [41], and allowing athletes to better tolerate greater training volumes with decreased subjective feelings of fatigue [42]. As β-alanine is considered primarily effective within 1- to 10-minute time frames [9], endurance athletes who undertake interval training sessions within that duration may benefit from an improved adaptive response to training by accumulating a greater workload during each session. This may be due to a number of inter-related factors including improved pH buffering [43], which can allow a greater reliance on aerobic metabolism and thus reduced accumulation of glycolytic metabolites for the same exercise intensity [44], decreased levels of blood lactate during the recovery from supra-maximal exercise [45], a reduction in oxidative stress due to the antioxidant effects of carnosine that could allow improved recovery between demanding workouts [46-48], an
increased threshold for neuromuscular fatigue [41], and/or reduced feelings of fatigue during periods of heavy training [42, 44]. It has also been suggested that increases in calcium sensitivity [49], or in calcium re-uptake [50], may also play a role in the ergogenic effects of β-alanine by helping to slow the decline in muscle performance during fatiguing exercise. From a cell-signaling perspective, β-alanine supplementation may impact mitochondrial biogenesis via increased calcium re-uptake [50] and its downstream effects on AMPK, CaMK, and extracellular-signal-regulated kinases (ERK) 1 and 2 [51], increased expression of peroxisome proliferator-activated receptor (PPAR)β/δ [52], and/or greater lactate signaling [24, 53] (Fig. 2).

Most studies have investigated the effects of β-alanine on exercise performance, with relatively few studies considering the effects of supplementation on adaptations to training (Table 1). Of the studies that have measured changes in VO$_{2peak}$ after endurance training, none have reported greater improvements from supplementation compared with the placebo groups [53-57]. This finding is similar to sodium bicarbonate and would be expected as buffering agents are more likely to exert their effects in the skeletal muscle rather than through central mechanisms. However, similar increases in cytochrome-c oxidase and β-hydroxyacyl-CoA dehydrogenase (β-HAD) maximal activities were found in active but untrained men taking β-alanine or a placebo during six weeks of Sprint Interval Training (SIT) (three sessions per week) [55], suggesting differences in peripheral adaptations may not be seen when total work performed is the same. This is in contrast to some findings for sodium bicarbonate [33, 34].
Performance improvements with β-alanine supplementation, compared with a placebo, have been reported for 10-km time-trial running performance (~54 min) following three weeks of run training [58], and cycling time-to-exhaustion at 120% of peak power (~3.5 min) following a five-week SIT program [53] (Table 1). There was also a trend for greater improvement in cycling time-to-exhaustion at 110% of peak power (~23 min) after six weeks of HIIT [57]. In contrast, four to six weeks of interval training in recreationally-active participants taking β-alanine or a placebo resulted in similar improvements for 250-kJ (~20 min) time-trial performance [55], average power during a 4-min time-trial [59], power at the ventilatory threshold [54], and critical power during a 3-minute all-out cycling test [56].

Considering its established effects on high-intensity exercise performance [9], it is challenging to distinguish the effects of β-alanine on performance from its effects on adaptations to training. However, as shown in Figure 4, there appear to be both independent and additive benefits of SIT and β-alanine supplementation.

One of the proposed mechanisms for β-alanine to improve training adaptations is an increased training intensity. This was observed in one study that showed improvements [53], while a trend for increased training intensity was seen in another that also showed favorable improvements [57]. Furthermore, there were increased blood lactate levels during sprint intervals with supplementation, compared with a placebo [53, 60]. Although training intensities were not reported in two other studies that showed improvements, they used interval-training protocols that elicited maximal, rather than pre-determined, effort levels [56, 58]. No differences in training intensity were seen in studies that showed no additional improvements from β-alanine either with maximal (30-s) sprint training [55] or sprints at
pre-determined work intensities [54, 59, 61]. This suggests greater training adaptations with
β-alanine supplementation are less likely to be observed if training intensities are
intentionally clamped or the chosen interval prescriptions are outside of the 1 to 10 minute
range of exercise where β-alanine exerts its most prominent influence [62]. With typical
dosing regimens of 3.2 to 6.4 g/d, at least two weeks of supplementation is needed to elevate
muscle carnosine concentrations [40, 63], before potentially exerting beneficial effects on
training intensity. Therefore, muscle carnosine loading may be beneficial prior to
undertaking an exercise training study.

2.3 Summary

The use of sodium bicarbonate (which causes an acute change in blood buffering capacity)
or β-alanine (which causes a chronic change in muscle buffering capacity) show potential for
allowing greater adaptations to training, though the data are mixed. Contrasting results may
be due to differences in training status, training intensity, or the use of interval prescriptions
that clamp exercise intensities and/or may fall outside of the optimal duration for the
supplement to be effective (e.g. <60 s). Furthermore, responses to sodium bicarbonate
appear subject to both intra-[31] and inter-[64] individual variability, at least in untrained
participants. Although the performance effects of co-ingesting sodium bicarbonate and β-
alanine are unclear [65], several studies have investigated the effects of combined
supplementation on training adaptations [66-68]. While a 4-wk loading period of β-alanine
supplementation is typically used when studying co-ingestion, in all but one study [61]
participant training sessions were not monitored. As discussed, differences in training
intensity appear necessary to see beneficial training adaptations with β-alanine supplementation.

Research using doses that have been shown to increase blood pH or muscle carnosine content should investigate the longer-term effects of sodium bicarbonate and β-alanine supplementation on training capacity to determine if athletes can increase their training volume, which could allow greater training adaptations and, in turn, better performance, as well as the effects of training status on training adaptations. Particularly for β-alanine, interval prescriptions should avoid pre-determined workloads (e.g. 4 min at 90% of peak power), in favor of distance or time-based intervals (e.g. 1-km, or 4-min maximal efforts). If set workloads are required, using a percentage of lactate threshold may be preferable to a percentage of VO_{2peak}. In addition to work completed during each session, measurement of blood lactate concentrations may be useful in determining if changes in lactate levels, compared with a placebo, can be predictive of an augmented training response (Table 2).

3. Dietary Nitrate

Dietary nitrate (NO_3^-), commonly supplemented and studied in the form of beetroot juice, has the potential to augment adaptations to endurance training due to its ability to increase plasma levels of nitrite (NO_2^-) and nitric oxide (NO) [69]. It can increase training intensity [70] (and thus total amount of work completed in a workout) by enhancing mitochondrial efficiency [71], reducing the oxygen cost of muscle contraction [72], and increasing contractile force in fast-twitch muscles [73]. Increases in mitochondrial biogenesis (via NO stimulation of guanylate cyclase leading to activation of PGC-1α) [74], and a shift in muscle
fibre type towards a more oxidative phenotype when combined with SIT (via nitric oxide’s role in calcineurin–NFAT [nuclear factor of activated T-cells] signaling) have also been reported [75-77], which are possibly related to increases in mitochondrial hydrogen peroxide [78]. However, by reducing the oxygen cost of muscle contraction, thereby reducing the metabolic stress inside the muscle, it is also possible that nitrate supplementation may impair training adaptations via decreased activation of cell-signaling pathways (e.g. AMPK, CaMK, and PGC-1α) [79].

The majority of studies showing beneficial effects of supplementation on endurance performance have used doses of 6 to 8 mmol NO₃⁻ taken as a single dose 2 to 3 hours prior to exercise or with a loading period of 5 to 8 days [10], although higher doses (e.g. 8 to 12 mmol) may be required for elite athletes [80]. Plasma levels of NO₂, used as a marker of NO availability [81], are increased in a dose-dependent manner but benefits may plateau between 8 and 16 mmol NO₃⁻ [82]. No effects of beetroot juice were observed after six weeks of cycling HIIT when ingesting ~5 mmol NO₃⁻ per day [83], while training studies using 8 to 12.8 mmol NO₃⁻ per day have shown benefits [75, 84-86].

Seven studies have supplemented dietary nitrate while undergoing supervised endurance training for at least three weeks [75, 77, 83-87] (Table 3). Four weeks of SIT combined with beetroot juice supplementation resulted in training-induced improvements in VO₂peak of 7.7 to 10.7% [85, 75], which were greater than with SIT alone (+5.6%) or after SIT with potassium NO₃⁻ (KNO₃) supplementation (+4.2%) [85]. Time-to-fatigue during high-intensity cycling (power at the gas exchange threshold plus 85% of the difference between
that work rate and VO2peak) also improved more with SIT+ beetroot juice (+71%) compared to SIT alone (+47%) and SIT+KNO3 (+42%) [85]. Greater reductions in blood pressure were observed with SIT+ beetroot juice compared with SIT+KNO3, a disparity that has also been reported between the ingestion of beetroot juice and NaNO3 [88] and may be related to the presence of other bioactive compounds found in beetroot juice [89]. Though speculative, the greater decrease in plasma NO2− observed during exercise with SIT+ beetroot juice compared with SIT+KNO3 may suggest enhanced NO synthesis during exercise, and is in accordance with observed correlations between the decline in plasma NO2− and improvements in exercise performance [90]. Two of the training studies used NaNO3 as the source of nitrate (6.5 and 11 mmol per day), and neither found any additional performance improvements [77, 87]. Further research should examine the effects of supplementation with different dietary nitrate sources on adaptations to endurance training.

Similar to buffering agents, dietary nitrate works primarily on peripheral metabolism and short-term intake would not be expected to have an appreciable influence on VO2peak. One explanation for the observed improvements in VO2peak may be related to changes in muscle fibre type. The percentage of slow-twitch fibres is directly related to VO2peak and endurance performance [91, 92], and the two studies that showed an additional increase in VO2peak with supplementation also found changes in fibre-type composition [75, 85]. A decreased proportion of type IIx muscle fibres in the vastus lateralis was observed in the nitrate group compared with placebo [75], and there was a greater increase in the proportion of type IIa fibres after both SIT alone (+20%) and SIT+ beetroot juice (+14%), compared with SIT+KNO3 (non-significant decrease) [85]. It is unclear if these differences suggest that
SIT+KNO is not as effective as SIT alone, or if this highlights the variability and/or reliability of fibre-type measures, which are reported to have inter-biopsy coefficient of variations of 21.5, 15.4 and 42.0 % for type I, Ila and IIX fibres, respectively [93]. Five weeks of SIT in hypoxia with NaNO₃ supplementation also increased the relative number of type IIA fibres, which was not observed with placebo supplementation in either normoxia or hypoxia, although similar improvements between groups were seen in VO₂max, the lactate threshold, and CS activity [77]. This is in accordance with in vitro research showing nitrate increased PGC-1α gene expression and a switch toward type I and IIA muscle fibres [94].

Two studies have examined the effects of dietary nitrate on training adaptations one to seven days after stopping supplementation [95, 87]. Twenty-eight days of NO₃⁻ supplementation in recreationally-active participants who maintained their typical exercise habits was followed by exercise testing after one day of placebo or continued nitrate in order to study chronic + acute vs. chronic-only dosing [95]. There was a reduction in the oxygen cost of submaximal cycling even up to 24 h after consuming the final dose of NO₃⁻, which was similar to what was observed after 28 days of supplementation followed by an acute dose of NO₃⁻ prior to testing [95]. There are several potential reasons for this observation, including lasting improvements in mitochondrial [71] or muscle contractile efficiency [72] from chronic supplementation, or that in spite of plasma nitrite concentrations returning to baseline after 24 h NO bioavailability remained elevated as a result of stored NO₂⁻ and NO₃⁻ in skeletal muscle [96]. Another study investigated three weeks of high-volume HIIT in recreationally-active participants consuming either ~11 mmol NaNO₃⁻ per day or a placebo, who performed a series of exercise tests two to seven days after the final day of supplementation [87]. Total
oxygen consumption decreased by 5% in the supplement group but not the placebo group during a time to exhaustion test at 80% $W_{\text{max}}$ that was performed 4 to 5 days after cessation of supplementation, although no differences were seen between groups for improvements in the time itself or in VO$_2$peak$^{[87]}$. Overall, limited data suggest the beneficial effects of NO$_3^-$ supplementation on oxygen consumption during submaximal exercise may continue to be observed for up to 5 days, but mechanisms and the exact time-course of washout remains to be elucidated.

3.1 Summary

The limited evidence suggests there may be small but favourable effects of endurance training with nitrate supplementation, which are possibly related to changes in muscle fibre-type. Beetroot juice may be more effective than nitrate salts, though the efficacy of supplementation can be affected by inter-individual variability$^{[97]}$ and environmental conditions$^{[98]}$. All studies to date have used high-intensity training protocols, as dietary nitrate is particularly effective at augmenting physiological responses in type II fibres$^{[99]}$. Studies using other forms of endurance training are needed to differentiate acute ergogenic benefits from the chronic effects of dietary nitrate on training adaptations. More research is required to determine the role of nitric oxide on mitochondrial biogenesis$^{[74]}$, as well as to investigate differences in the skeletal muscle remodeling responses - particularly between untrained and endurance-trained participants (Table 2).

4. Antioxidants
Free radicals are produced during aerobic metabolism and play a role in both exercise-induced fatigue and the adaptive response to exercise [100]. Muscle contractile force is decreased by elevated levels of reactive oxygen and nitrogen species (RONS), largely due to changes in myofibrillar calcium sensitivity [101]. At the same time, RONS generated during exercise act as signaling molecules to increase the production of proteins involved in the skeletal muscle adaptation to exercise such as NO synthase, superoxide dismutase (SOD), and MAP kinases p38, ERK1 and ERK2 (Fig. 2) [102, 103]. For example, RONS, via AMPK activation, can induce PGC-1α promoter activity and mRNA expression [104]. Due to their effects of reducing RONS, antioxidants have the potential to impair the adaptive responses to RONS-induced stressors and therefore negate favourable training adaptations that would normally occur [105], while also having the potential to improve recovery and acute performance [106-108]. Some antioxidants, including polyphenols, may exert their effects in areas beyond RONS signaling, such as increased mobilisation of fatty acids [109] or sirtuin 1 (SIRT1) activation (Fig. 2) [110]. For additional discussion of the effects of antioxidant supplementation on exercise performance the reader is referred to recent reviews [14, 105, 111].

4.1 Vitamins C and E

Vitamins C and E are antioxidant vitamins with a role in protecting cellular organelles from oxidative damage [112, 113]. No impact on exercise performance outcomes has been observed in human studies when supplementing with vitamins C and/or E during controlled endurance training [114-125] (Table 4). Skeletal muscle adaptations, such as training-induced increases in CS or β-HAD enzyme activity, mRNA or protein levels of cytochrome c
oxidase subunit IV (COX-IV), heat shock protein 70, or changes in substrate oxidation, have not been affected by supplementation in healthy participants after 4 to 12 weeks of training [120, 123-126]. In contrast, supplementation with vitamins C and E for 4 to 11 weeks has blunted training-induced increases in COX-IV, PGC-1α, PGC-1β, PPARγ, SOD, and GPx protein compared with a placebo in both untrained and previously-trained participants [122, 127]. Animal studies have also shown that 4 to 14 weeks of aerobic training with antioxidant intake blunted training-induced increases in cytochrome oxidase and CS activity, mitochondrial transcription factor A (TFAM), CYT-C, GPx, glutathione reductase, SOD, PGC-1α, NRF-1, and NRF-2 [118, 128-130], although other research has shown no effect on markers of training adaptations such as mitochondrial respiratory capacity, and CYT-C, COX-I, COX-IV, CS, NRF-1, or PGC-1α protein content [130-133].

The reason for these divergent findings, particularly in the animal research, may be due to variations in dosing, exercise protocols, and baseline levels of endogenous antioxidants. The amount of antioxidant provided has varied more than 10-fold [128, 134], exercise type has included both running and swimming, and the duration of training sessions has ranged from 30 min to six hours per day [132-134]. Potential redundancies in the adaptation process must also be considered. For example, contracting skeletal muscle activates AMPK via its upstream kinase Ca2+/calmodulin-dependent protein kinase kinase (CaMKK) [135], and changes in proteins regulating fat metabolism such as FAT/CD36 are mediated in part by contraction-induced signaling of the ERK1/2 pathway [136]. Thus, many of the common adaptations to endurance training may be observed in the absence of RONS signaling and be minimally influenced by antioxidant supplementation.
It should also be considered that some effects may be missed due to small sample sizes. For example, the two human studies showing a blunting of training adaptations with antioxidant supplementation also had the largest sample sizes [122, 127]. In contrast, eight weeks of endurance training with or without supplementation had no statistical effect on increases in VO\textsubscript{2max}, but the vitamin C group (n=5) improved by 10.8% while the group without supplementation (n=9) improved by 22% (d = -1.0) [118].

The effects of vitamins C and E supplementation on markers of oxidative stress have also been variable, with reports of reductions [116], increases [119, 137], and no impact [123, 138]. This may be primarily related to methodological issues including the use of assays that are no longer recommended, short half-lives of oxidants being measured, and lack of measurement specificity, making both conducting and interpreting the available research difficult [139-141]. More research is needed before a conclusion can be drawn regarding the impact of vitamins C and E on markers of oxidative stress during endurance training.

4.2 Polyphenols

Polyphenols are antioxidants found in plants, with the potential to impact chronic adaptations to endurance training by several mechanisms that differ from the antioxidant effects of vitamins C and E. Rather than acting as scavengers, they can stimulate stress-related cell signaling pathways and increase the expression of genes encoding proteins, such as NRF2, as well as by stimulating the SIRT1-AMPK-PGC1\textalpha pathway in skeletal muscle, leading to increased mitochondrial biogenesis (Fig. 2) [142]. Perhaps, paradoxically for the
endurance athlete, it is thought that AMPK activation relies on the ability of polyphenols, such as quercetin and resveratrol, to directly bind and inhibit the mitochondrial F1F0-ATPase/ATP synthase (Complex V), thus impairing ATP production [143]. A number of studies have investigated the effect of polyphenols on exercise performance [142]; however, there are few that have also included supervised endurance training programs (Table 4).

4.2.1 Resveratrol

Resveratrol is a polyphenol found in grapes, red wine, and other plant species and is known to activate SIRT1 [110], and can shift muscle fibres towards a more oxidative phenotype [144]. Resveratrol supplementation during eight weeks of HIIT in sedentary men blunted the training-induced improvements in VO2max and protein carbonylation, while having no effect on PGC-1α mRNA, CYT-C, CS and β-HAD activity, or time to exhaustion during a one-legged knee-extensor test, compared with a placebo [145, 146]. In contrast, four weeks of resveratrol supplementation by recreationally-active men performing HIIT resulted in no differences in training-induced increases in VO2max, peak aerobic power, Wingate power, succinate dehydrogenase activity, or fibre-type distribution, compared to placebo, although the resveratrol group had smaller increases in the gene expression of PGC-1α, SIRT1, and SOD [147]. This is in line with in-vitro research showing acute exposure to resveratrol inhibits AMPK activity in human skeletal muscle cells [148]. Following 12 weeks of treadmill training with resveratrol, rats bred for increased endurance capacity had increased activation of the AMPK-SIRT1-PGC-1α pathway and VO2max [149], while rats bred for low aerobic capacity had no differences in VO2max, AMPK, or SIRT1 levels [150], suggesting the response to resveratrol may be influenced by training status. The available data on
resveratrol are limited but suggest that active/trained humans and animals may respond differently than those who are sedentary/untrained.

4.2.2 Green Tea Extract

Green tea extracts are a type of polyphenolic flavonoids which, beyond their role as an antioxidant, may also play a role in the mobilisation and oxidation of fatty acids [151] that appears to be mediated, at least in part, by reducing the malonyl-CoA content in skeletal muscle [152]. Supplementing green tea extract during ten weeks of moderate-intensity endurance training in healthy males resulted in greater fat oxidation while cycling at 55% VO$_2$peak, with no changes observed in the placebo group [109] (Table 4). Untrained men who performed a 4-week training intervention with green tea extract or placebo had similar improvements in VO$_2$max and run to exhaustion time (8.1–9.7 km/h at 18-20% grade), with no differences between groups for total antioxidant status [153]. However, these are performance tasks that would not be expected to benefit from increased fat oxidation rates as they are at an intensity that would be reliant on carbohydrate oxidation [154]. Mice that underwent endurance training for 10 weeks with green tea extract supplementation had better running (~2.5 h) and swimming (~40 min) times to exhaustion compared with the exercise-only group, along with higher levels of muscle β-oxidation and FAT/CD36 mRNA [152, 155]. The limited data examining green tea extract in the context of endurance training support the notion of increased fat oxidation and improvement in exercise time to exhaustion in animals but not untrained men; however, studies that include other measures of performance (e.g. time-trials) are needed, particularly at intensities and durations that may favour improved fat oxidation.
4.3 Other Antioxidants

Supplementation with (-)-epicatechin, a component of cocoa known to activate the SIRT1 pathway [156], impaired training adaptations during four weeks of cycling training; increases in VO$_{2\text{peak}}$ (+22.6%) and succinate dehydrogenase activity (+59.1%) were seen in the placebo but not the supplement group, although no differences in peak power or CS activity were observed between groups [157] (Table 4). In contrast, mice undergoing treadmill training had greater increases in time to exhaustion, CS levels, and protein levels of PGC-1β and TFAM with (-)-epicatechin supplementation compared with exercise-only [158]. The use of allopurinol (an inhibitor of xanthine oxidase) in rats for six weeks had no effect on training-induced increases in PGC-1α, TFAM, or CYT-C protein expression, or CS and β-HAD enzyme activity, despite an attenuation of key signaling proteins (p38 MAPK, ERK1/2, and TFAM) after a single session of exercise [103, 159], highlighting the importance of studying longer-term adaptations to endurance training.

4.4 Summary

Endurance-related performance improvements following one- to six-month training programs are not impacted by supplementation with vitamin C and/or vitamin E and are unlikely to be affected by other antioxidants, although fewer data are available. Less clear are the effects of vitamins C and E on markers of training adaptations and oxidative stress. The disconnect between an impaired adaptive response without measurable performance decrements may imply that longer time frames are needed before observable differences in performance can be detected. Differences in training protocols, training status, and type and
amount of supplementation, may also be responsible for contrasting results, as well as inherent redundancy in the mechanisms governing skeletal muscle adaptations to exercise. ROS production is only one of the mechanisms by which adaptation is regulated, along with contraction-induced changes in mechanical strain, ATP turnover, calcium flux, redox balance, and intracellular oxygen pressure (Fig. 2) [3]. Although many studies report attenuated levels of signaling molecules involved in mitochondrial biogenesis, increased levels of CS activity suggest that changes in mitochondrial volume are not inhibited by antioxidant supplementation [123, 159]. Future research should attempt to differentiate between the antioxidant actions of a given supplement and other signaling pathways (e.g. AMPK, SIRT1) that may be influenced.

5. Caffeine

Caffeine is well-studied and commonly reported to exhibit performance-enhancing effects during endurance exercise [160]. When taken in moderate doses (3 to 6 mg/kg), 2 to 3% improvements are seen in time-trial performance lasting 6 min to 2.5 h [160] and power during HIIT [161]. It has been suggested that habitual caffeine usage will decrease its efficacy [162]. However, caffeine supplementation (4 to 6 mg/kg) has improved 30- to 45-min cycling time-trial performance in habitual low, moderate, and high caffeine consumers [163, 164], and the ingestion of 3 mg/kg for 20 days continued to produce an ergogenic effect [165], suggesting that regular caffeine consumption during training sessions should not reduce its longer-term impact. Despite its widespread use, little is known about the effects of caffeine ingestion prior to exercise on subsequent adaptations to training.
There are several mechanisms that could lead to enhanced endurance training adaptations. By way of reduced perception of exertion, likely through its effects as an adenosine receptor antagonist [166], an athlete may be able to accumulate a greater training stress. Indeed, participants who consumed 3 to 6 mg/kg caffeine completed 12.6% more work during 30 minutes of cycling at a given level of perceived exertion [167], increased power by 2.8% during HIIT (8x5 min at maximum intensity with 1 min recovery) [161], and were able to mitigate reductions in power during training sessions performed with low carbohydrate availability [161]. Caffeine can increase exogenous carbohydrate oxidation [168], possibly via greater intestinal absorption [169], and also increase AMPK activation via calcium-mediated protein phosphatase 2A activity [170]. Emerging research also points to a role for caffeine in improving mitochondrial respiration via its effects on mitochondrial p27 [171], suggesting the potential to work synergistically with SIT - the form of interval training that has been associated with the greatest improvements in mitochondrial respiration [172].

Taken together, caffeine has the potential to impact training adaptations across a number of key pathways that can ultimately lead to greater increases in mitochondrial biogenesis (Fig. 2).

Despite the above rationale, only one study in humans, and one study in rats, has investigated the effects of caffeine on adaptations to endurance training. In the human study, the caffeine was part of a multi-ingredient supplement, dietary caffeine intake was not controlled for, and there were no differences in caffeine consumption between groups [173], while rats given caffeine prior to each exercise session during six weeks of HIIT were only studied for changes in brain and behavioral biomarkers [174]. Research should investigate the effects
of caffeine on training adaptations, in conjunction with the impact of exercise intensity, training status [175], sex [176], and genotype [177], using moderate (3 to 6 mg/kg) and low (<3 mg/kg) [178] doses.

6. Creatine

Though creatine supplementation is rarely associated with endurance sports, it has been shown to enhance exercise performance across a variety of sporting events (e.g. sprinting, middle-distance, team sports, and HIIT in endurance sports [179]) and this has been attributed to elevated muscle content of creatine and phosphocreatine [179]. A dose of 5 g four times per day for 5 to 7 days is commonly used as a loading protocol, with subsequent daily doses of 3 to 5 g/day to maintain elevated creatine levels [179]. However, high and low responders to creatine supplementation have been identified, possibly due to variations in baseline muscle creatine content [180], and/or its uptake and use [181]. Despite the accepted role of creatine for improving performance, there has been little investigation of its ability to affect adaptations to training.

There are several potential mechanisms by which creatine supplementation may be able to affect training adaptations. Exercise intensity appears to be a key factor for training-induced increases in mitochondrial respiration [172], and so any supplement that can increase exercise intensity could potentially offer additional benefit to endurance training, adaptation, and performance. In addition to its primary role of combining with a phosphoryl group to form phosphorylcreatine via the creatine kinase reaction, creatine may also play a role in aerobic energy metabolism by connecting sites of ATP production (glycolysis and
oxidative phosphorylation) with subcellular sites of ATP utilisation (ATPases) [182], with subsequent enhancement of mitochondrial respiration in slow-twitch but not fast-twitch fibres [183]. This may allow a greater amount of work to be completed during a training session, and a reduced oxygen cost during submaximal exercise. Creatine has also been shown to have antioxidant actions reducing the formation of ROS, oxidative DNA damage, and lipid peroxidation after exercise, which may affect exercise-induced cell signaling [184]. There is a potential for increases in body mass due to water retention with creatine loading [179]. However, despite a ~2.5% increase in body mass, well-trained cyclists undergoing both creatine and carbohydrate loading reported a greater power output during sprint efforts within a simulated 120-km time trial, with no differences in simulated uphill cycling, compared to placebo [185].

Two studies using recreationally-active men performing four weeks of HIIT while supplementing with either creatine (10 g/day) or a placebo reported significant improvements in the creatine but not placebo groups for critical power and ventilatory threshold (Table 5) [186, 187]. However, no differences were seen between groups for VO$_{2\text{peak}}$, time to exhaustion at VO$_{2\text{peak}}$, anaerobic working capacity, or total work done during a ride to exhaustion at 110% of peak aerobic power [186, 187]. Similar research in recreationally-active women found no differences in exercise-induced improvements in VO$_{2\text{peak}}$, ventilatory threshold, or 2-km time-trial performance (~2 min) [188]. However, that study did not match menstrual phase, which has been shown to impact the blood lactate response to high-intensity exercise [189]. Similar to other supplements, it is difficult to separate the effects of creatine on performance from its effects on adaptations to training.
For example, in the absence of training, 5 to 7 d of creatine loading resulted in increases in power at the lactate threshold [190] and supra-maximal time to exhaustion [190, 191], as well as lower VO₂ during sub-maximal cycling [191]. Thus, while creatine may improve some adaptations to endurance training, the limited evidence does not yet support its widespread use by endurance athletes. More research is needed, particularly in trained participants performing 15-s to 4-min intervals using maximal efforts rather than pre-determined work rates, while also controlling for the effects of the supplement alone (Table 2).

7. Conclusion

The most important variables impacting adaptations to endurance training are the training stimuli - volume and intensity. However, within a given training paradigm, the appropriate use of dietary supplements may offer additional benefits. These benefits appear largely by allowing increasing training intensities, and so HIIT prescriptions should avoid pre-determined workloads in favor of time-based intervals (e.g. 4-min maximal efforts). Beetroot juice appears to impact skeletal muscle fibre-type remodeling, though more research with concurrent endurance training is needed. Supraphysiological doses of antioxidants should be used with caution as research is unclear regarding the extent of their effects on training adaptations, but they appear unlikely to negatively affect performance in the short-term (< 6 months). Caffeine and creatine have potential for augmenting adaptations to endurance training, although clear data are lacking.

Finally, it is imperative for athletes who compete under anti-doping rules to avoid supplements that contain prohibited substances [192]. It is tempting to interpret small or
trivial effects in sport science research as unlikely to harm, but the risk of positive doping tests should be weighed against the unproven effects of a supplement. Clearly, strong caution must be used when athletes, coaches, and health care practitioners are selecting dietary supplements for use.
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Training Status</th>
<th>Length</th>
<th>Supplement Dosage</th>
<th>Type of Training</th>
<th>Total Number of Training Sessions</th>
<th>Performance (compared with placebo)</th>
<th>Adaptations (compared with placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edge et al. 2006[33]</td>
<td>16 f</td>
<td>Recreationally Active</td>
<td>8 weeks</td>
<td>0.4 mg/kg body mass</td>
<td>HIIT 3x/wk; 6-12x 2-min cycle intervals at 140 – 170% of LT, 1 min RBI</td>
<td>24</td>
<td>Lactate threshold (LT): +26 vs. 15% (d= 0.5, [95% CI -0.5, 1.5])</td>
<td>No differences in muscle buffer capacity</td>
</tr>
<tr>
<td>Driller et al. 2013[36]</td>
<td>12 m</td>
<td>Well-Trained</td>
<td>4 weeks</td>
<td>0.3 g/kg body mass</td>
<td>HIIT 2x/wk; 8 x 2.5-min rowing intervals at 90% of PPO, 3 min RBI</td>
<td>8</td>
<td>No differences in performance or LT</td>
<td>No difference in or VO$_{2}$peak</td>
</tr>
<tr>
<td>Hawke et al. 2014[35]</td>
<td>19 m</td>
<td>Recreationally Active</td>
<td>6 weeks</td>
<td>0.4 g/kg body mass</td>
<td>HIIT 3x/wk; 8-12x 2-min cycling intervals at 85-110% VO$_{2}$peak, 1 min RBI</td>
<td>18</td>
<td>Greater improvements in Wingate relative peak power (20.8 vs. 10.3% (d = 2.1, [95% CI 1.0, 3.2]))</td>
<td>Increased peak lactate concentration only in the sodium bicarbonate group</td>
</tr>
<tr>
<td>Wang et al. 2019[34]</td>
<td>20 m</td>
<td>Recreationally Active</td>
<td>6 weeks</td>
<td>0.2 g/kg body mass</td>
<td>HIIT 3x/wk; 4 sets of 20-30 s cycling at 100% PPO, 30-40 s at 70% PPO, 1 min RBI</td>
<td>18</td>
<td>Greater improvements in Wingate relative mean power</td>
<td>No differences in Wingate relative mean power</td>
</tr>
<tr>
<td>Bishop et al. 2010[37]</td>
<td>21 m rats</td>
<td>N/A</td>
<td>5 weeks</td>
<td>0.05 g/kg body mass</td>
<td>HIIT 5x/wk; 7-12 x 2 min treadmill intervals, 1 min RBI</td>
<td>25</td>
<td>Time to fatigue: 12-fold vs. 8-fold longer than control (d = 11.1, [95% CI 6.8, 15.3])</td>
<td>Greater improvements in mitochondrial mass and respiration (d= 1.8, [95% CI 0.6, 3.1])</td>
</tr>
<tr>
<td>Bellinger et al. 2012[61]</td>
<td>14 m</td>
<td>Well-Trained</td>
<td>4 weeks</td>
<td>65 mg/kg body mass/day</td>
<td>HIIT 2x/wk; 8 x 2.5 min cycling intervals at 90% VO$_{2}$max HR</td>
<td>8</td>
<td>No differences in 4-min time-trial power</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Overview of studies using buffering agents during supervised endurance training
<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>Status</th>
<th>Interventions</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Howe et al. 2013 [59]</td>
<td>16 m</td>
<td>Well-Trained</td>
<td>4 weeks, 4.5 g/day, HIIT 2x/wk; 8x 2.5 min at 90% PPO, 3 min RBI</td>
<td>No differences in 4-min time-trial power, trend for increased total work done (p=0.09)</td>
</tr>
<tr>
<td>Cochran et al. 2015 [55]</td>
<td>24 m</td>
<td>Recreationally Active</td>
<td>6 weeks, 4 weeks loading with 3.2 g/day, followed by 6 weeks of 3.2 g/day with supervised training, SIT 3x/wk; 4–6 Wingate tests, 4 min RBI</td>
<td>No difference in VO$_{2\text{peak}}$, repeated-sprint capacity, or time trial performance No difference in cytochrome-c oxidase or $\beta$-HAD maximal activities</td>
</tr>
<tr>
<td>Bellinger and Minahan 2016 [53]</td>
<td>14 m</td>
<td>Well-Trained</td>
<td>5 weeks, 4 weeks loading with 6.4 g/day, followed by 5 weeks of 1.2 g/day with supervised training, SIT 2x/wk; 4-6x 1-km (~1.3 min) cycling sprints, 4 min RBI</td>
<td>No difference in TT performance or VO$_{2\text{max}}$ Training intensity: +9.9 vs. 4.9% ($d=0.3$, [95% CI -0.8, 1.3]) Time to exhaustion at 120% PPO: +14.9 vs. 9.0% ($d=0.5$, [95% CI -0.6, 1.58])</td>
</tr>
<tr>
<td>Santana et al. 2018 [58]</td>
<td>16 m</td>
<td>Recreationally Active</td>
<td>23 days, 5 g/day, Moderate intensity 2x/wk (7-12 km), HIIT 1x/wk (6x500 m sprints, 2 min RBI)</td>
<td>10 km running performance: -6.7% vs. no change</td>
</tr>
<tr>
<td>Wang et al. 2018 [56]</td>
<td>38 m</td>
<td>Recreationally Active</td>
<td>4 weeks, 6.4 g/day, HIIT 2x/wk; three sets of 5x10 s maximal sprints, 20 s RBI, 5 min RBS</td>
<td>No difference in VO$_{2\text{max}}$ or PPO Anaerobic capacity: 14% higher than placebo ($d=0.8$, [95% CI -0.1, 1.8])</td>
</tr>
</tbody>
</table>

HIIT: High-intensity interval training, SIT: sprint-interval training, TTE: Time to exhaustion, PPO: Peak power output, RBI: Rest between intervals, RBS: Rest between sets

Effect size of supplement calculated as Cohen’s d when data were available (reported as effect size, 95% CI)
<table>
<thead>
<tr>
<th>Mechanisms of action</th>
<th>Ingestion</th>
<th>Type of training</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bicarbonate</td>
<td></td>
<td>Interval training sufficient to elevate lactate levels and/or pH compared with a placebo</td>
<td>- Lactate threshold&lt;br&gt;- Mitochondrial mass and respiration&lt;br&gt;- Time to fatigue at maximal or near-maximal efforts&lt;br&gt;- Time-trial performance</td>
</tr>
<tr>
<td>- Increased training intensity&lt;br&gt;- Increased pH --&gt; improved mitochondrial adaptations</td>
<td>0.2-0.4 g/kg body weight - sufficient to raise blood [HCO3⁻] by 5-6 mmol</td>
<td>1 to 4 min intervals using maximal efforts rather than pre-determined work rates, sufficient to elevate blood lactate compared with a placebo</td>
<td>- Open-ended exercise tests (e.g. time to exhaustion) rather than fixed end-point tasks (e.g. time trials)&lt;br&gt;- Training intensity&lt;br&gt;- Oxidative stress</td>
</tr>
<tr>
<td>Beta-alanine</td>
<td></td>
<td>2-4 wk loading phase of 4-6 g/d, followed by 3-6 g/d</td>
<td></td>
</tr>
<tr>
<td>- Increased training intensity&lt;br&gt;- Increased pH --&gt; improved mitochondrial adaptations&lt;br&gt;- Improved Ca sensitivity&lt;br&gt;- Reduced lipid peroxidation</td>
<td></td>
<td>15 s to 4 min intervals using maximal efforts rather than pre-determined work rates, steady-state endurance</td>
<td></td>
</tr>
<tr>
<td>Dietary nitrate</td>
<td></td>
<td>8-13 mmol/d from beetroot juice</td>
<td></td>
</tr>
<tr>
<td>- Increased training intensity&lt;br&gt;- Increased mitochondrial biogenesis via nitric oxide signaling and possibly RONS&lt;br&gt;- Reduced oxygen cost&lt;br&gt;- Muscle fibre-type remodeling</td>
<td></td>
<td>- Mitochondrial mass and respiration&lt;br&gt;- Muscle fibre-type changes&lt;br&gt;- Training intensity&lt;br&gt;- VO₂max</td>
<td></td>
</tr>
<tr>
<td>Antioxidants</td>
<td></td>
<td>More research needed before recommendations can be made, particularly with regard to timing relative to exercise</td>
<td>- Mitochondrial adaptations&lt;br&gt;- Performance changes&lt;br&gt;- Training intensity</td>
</tr>
<tr>
<td>- Reduced oxidative stress leading to improved training and recovery&lt;br&gt;- Stimulate or impair stress-related signaling pathways&lt;br&gt;Stimulate mitochondrial biogenesis</td>
<td></td>
<td>More research needed before recommendations can be made</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
<td>2-6 mg/kg</td>
<td></td>
</tr>
<tr>
<td>- Increased training intensity via decreased perception of exertion&lt;br&gt;- Improved Ca sensitivity&lt;br&gt;- Increased AMPK signaling</td>
<td></td>
<td>0.5 to 4 min intervals using maximal efforts rather than pre-determined work rates</td>
<td>- Mitochondrial mass and respiration&lt;br&gt;- Training intensity</td>
</tr>
<tr>
<td>Creatine</td>
<td></td>
<td>10 g/d for 10 d, then 10 g/d on training days only - more research needed</td>
<td></td>
</tr>
<tr>
<td>- Increased training intensity&lt;br&gt;- Improved energy production&lt;br&gt;- Reduced lipid peroxidation</td>
<td></td>
<td>15 s to 4 min intervals using maximal efforts rather than pre-determined work rates</td>
<td>- Mitochondrial respiration&lt;br&gt;- Training intensity&lt;br&gt;- Time-trial performance</td>
</tr>
<tr>
<td>Participants</td>
<td>Training Status</td>
<td>Length</td>
<td>Supplement Dosage</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------------</td>
<td>--------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>Puype et al. 2014[83]</td>
<td>22 m Moderately Trained</td>
<td>6 weeks</td>
<td>~5 mmol per day (0.07 mmol NO_3^-/kg body mass)</td>
</tr>
<tr>
<td>De Smet et al. 2016[77]</td>
<td>27 m Recreationally Active</td>
<td>5 weeks</td>
<td>6.5 mmol NaNO_3^-</td>
</tr>
<tr>
<td>Muggeridge et al. 2017[84]</td>
<td>27 m Recreationally Active</td>
<td>3 weeks</td>
<td>~8 mmol nitrate [0.06–0.15 mmol/kg body mass]</td>
</tr>
<tr>
<td>Thompson et al. 2017[75]</td>
<td>18 m, 18 f Recreationally Active</td>
<td>4 weeks</td>
<td>12.8 mmol NO_3^- per day (2 servings of 6.4 mmol, AM/PM)</td>
</tr>
<tr>
<td>Thompson et al. 2018[85]</td>
<td>18 m, 12 f Recreationally Active</td>
<td>4 weeks</td>
<td>12.8 mmol NO_3^- per day (2 servings of 6.4 mmol, AM/PM)</td>
</tr>
<tr>
<td>Finkel et al. 2018[87]</td>
<td>17 m Recreationally Active</td>
<td>3 weeks</td>
<td>~11 mmol per day (0.14 mmol NaNO_3^-/kg body mass)</td>
</tr>
<tr>
<td>Santana et al. 2019[86]</td>
<td>16 m Recreationally Active</td>
<td>4 weeks</td>
<td>~12 mmol per day (across three servings)</td>
</tr>
</tbody>
</table>

HIIT: High-intensity interval training, SIT: sprint-interval training, TTE: Time to exhaustion, PPO: Peak power output, RBI: Rest between intervals, Wmax: Maximal aerobic wattage

Effect size of supplement calculated as Cohen’s d when data were available (reported as effect size, 95% CI)
<table>
<thead>
<tr>
<th>Humans</th>
<th>Supplement</th>
<th>Supplement Dosage</th>
<th>Participants</th>
<th>Training Status</th>
<th>Length</th>
<th>Type of Training</th>
<th>Total Number of Training Sessions</th>
<th>Performance (compared with placebo)</th>
<th>Metabolic Adaptations (compared with placebo)</th>
<th>Oxidative Stress (compared with placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharman et al. 1971[114]</td>
<td>Vitamin E</td>
<td>400 mg</td>
<td>26 m</td>
<td>Trained</td>
<td>6 weeks</td>
<td>4x/wk Competitive swim training - unspecified</td>
<td>24</td>
<td>No difference in 1 mi run or 400 m swim times</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lawrence et al. 1975[115]</td>
<td>Vitamin E</td>
<td>900 iu</td>
<td>48</td>
<td>Well-trained</td>
<td>6 months</td>
<td>Competitive swim training - unspecified</td>
<td>Unspecified</td>
<td>No difference in 500 yd or 100 yd swim times</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rokitzki et al. 1994[116]</td>
<td>Vitamin E</td>
<td>330 mg</td>
<td>30 m</td>
<td>Well-trained</td>
<td>5 months</td>
<td>Competitive cycling training - unspecified</td>
<td>Unspecified</td>
<td>No difference in LT</td>
<td>Reduced MDA</td>
<td></td>
</tr>
<tr>
<td>Oostenbug et al. 1997[117]</td>
<td>Vitamin E</td>
<td>300 iu</td>
<td>24 m</td>
<td>Well-trained</td>
<td>3 weeks</td>
<td>Competitive cycling training - unspecified</td>
<td>Unspecified</td>
<td>No difference in VO_{2max}, maximal workload, or 1-hr time-trial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomez-Cabrera et al. 2008[118]</td>
<td>Vitamin C</td>
<td>1000 mg</td>
<td>14 m</td>
<td>Sedentary</td>
<td>8 weeks</td>
<td>3x/wk; 40 min steady-state cycling at 65-80% VO_{2max}</td>
<td>24</td>
<td>Trend for smaller improvement in VO_{2max}: +10.7 vs. 22% (d=-1.1, [95% CI -2.2, 0.04])</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomez-Cabrera et al. 2008[118]</td>
<td>Vitamin C</td>
<td>1000 mg</td>
<td>14 m</td>
<td>Sedentary</td>
<td>8 weeks</td>
<td>3x/wk; 40 min steady-state cycling at 65-80% VO_{2max}</td>
<td>24</td>
<td>Trend for smaller improvement in VO_{2max}: +10.7 vs. 22% (d=-1.1, [95% CI -2.2, 0.04])</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomez-Cabrera et al. 2008[118]</td>
<td>Vitamin C</td>
<td>1000 mg</td>
<td>15 m</td>
<td>Recreationally Active</td>
<td>4 weeks</td>
<td>4x/wk HIIT; 5x3 min treadmill running at 90% VO_{2max}, 3 min RBI</td>
<td>16</td>
<td>No difference in VO_{2max} or 10-km time-trial performance</td>
<td>No difference in substrate utilization</td>
<td></td>
</tr>
<tr>
<td>Braakhuis et al. 2014[119]</td>
<td>Vitamin C</td>
<td>1000 mg</td>
<td>23 f</td>
<td>Trained</td>
<td>3 weeks</td>
<td>3x/wk running; 4x 3-5 min and 6x2 min hill repeats</td>
<td>10</td>
<td>No difference in 5-km time trial</td>
<td></td>
<td>Increased protein carbonyl at rest, increased SOD post-exercise, blunting training-induced decrease in resting CAT activity</td>
</tr>
<tr>
<td>Bryant et al. 2009[121]</td>
<td>Vitamin C and E</td>
<td>1000 mg vit C, 400 iu/kg vit E, or 1000 mg vit C + 200 iu/kg vit E</td>
<td>7 m</td>
<td>Trained</td>
<td>3 weeks</td>
<td>Competitive cycling training, 250–350 km/wk</td>
<td>Unspecified</td>
<td>No difference in work completed</td>
<td>No difference in substrate oxidation</td>
<td>Vit C Increased MDA, no difference with vit C+E</td>
</tr>
<tr>
<td>Ristow et al. 2009[117]</td>
<td>Vitamin C and E</td>
<td>1000 mg vit C, 400 iu vit E</td>
<td>39 m</td>
<td>Trained and Untrained</td>
<td>4 weeks</td>
<td>5x/wk; 20 min biking or running, 45 min circuit training</td>
<td>20</td>
<td>Decreased PGC-1a, PGC-1β, PPARγ, SOD, and GPx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Treatment</td>
<td>Dose</td>
<td>Gender</td>
<td>Exercise Protocol</td>
<td>Duration</td>
<td>VO2max, Maximal Workload, or LT Changes</td>
<td>CS, β-HAD, PGC-1a, or PPARγ Changes</td>
<td>SOD Changes</td>
<td>GPx, SOD, GSH, or HSP70 Changes</td>
<td></td>
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<td>-------------------------------------------</td>
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</tr>
<tr>
<td>Yfanti et al. 2010,123, 2011,124</td>
<td>Vitamin C and E</td>
<td>500 mg vit C, 400 iu vit E</td>
<td>21 m</td>
<td>5x/wk cycling; one day each of graded exercise test; 10x3 min at 85% PPO; 3 min RBI; 1x60 min at 60% PPO; 5x8 min at 75% PPO; 4 min RBI; 1x120 min at 55% PPO</td>
<td>12 weeks</td>
<td>No difference</td>
<td>No difference in CS, B-HAD, PGC-1a, or PPARγ</td>
<td>No difference in SOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paulsen et al. 2014,122, Cumming et al. 2014,126</td>
<td>Vitamin C and E</td>
<td>1000 mg vit C, 235 mg vit E</td>
<td>26 m, 28 f</td>
<td>3-4 w/wk running; 2 days steady state 30-60 mins at 72-87% HR max, 2 days 4-6 x 4-6 min &gt;90% HR max</td>
<td>11 weeks</td>
<td>No difference in VO2max or shuttle run test</td>
<td>Decreased PGC-1α and COX-IV</td>
<td>Decreased uric acid</td>
<td>No difference in GPx, SOD, GSH, or HSP70</td>
<td></td>
</tr>
<tr>
<td>Morrison et al. 2015,125</td>
<td>Vitamin C and E</td>
<td>1000 mg vit C, 400 iu vit E</td>
<td>11 m</td>
<td>3x/wk cycling, 10x4 min at 90% VO2max, 2 min RBI</td>
<td>4 weeks</td>
<td>No difference in VO2max</td>
<td>No difference in CS or COX-IV</td>
<td>Decreased SOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shill et al. 2016,193</td>
<td>Coenzyme Q10</td>
<td>10 mg MitoQ (proprietary CoQ-10)</td>
<td>20 m</td>
<td>3-5x/wk cycling, 45-60 min at 50-70% VO2max</td>
<td>3 weeks</td>
<td>No difference in VO2max</td>
<td>No difference in mitochondrial function</td>
<td>No difference in MDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ichinose et al. 2011,109</td>
<td>Green tea extract</td>
<td>578 mg tea catechins</td>
<td>20 m</td>
<td>3x/wk cycling, 60 min at 60% VO2max</td>
<td>10 weeks</td>
<td>No difference in VO2max</td>
<td>Increased fat utilization</td>
<td>No difference for MDA or total antioxidant status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuo et al. 2015,153</td>
<td>Green tea extract</td>
<td>207 mg tea catechins</td>
<td>40 m</td>
<td>3x/wk running, 20 min at 75% oxygen uptake reserve</td>
<td>4 weeks</td>
<td>No difference in VO2max or time to exhaustion</td>
<td>No difference in Succinate dehydrogenase activity</td>
<td>Decreased gene expression of PGC-1a, SIRT1, and SOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scribbans et al. 2014,147</td>
<td>Resveratrol</td>
<td>150 mg</td>
<td>16 m</td>
<td>3x/wk HIIT cycling, 8x20 s at 170% PPO, 10 s RBI</td>
<td>4 weeks</td>
<td>No difference in VO2max, peak aerobic power, or Wingate power</td>
<td>No difference in succinate dehydrogenase activity</td>
<td>Decreased gene expression of PGC-1a, SIRT1, and SOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliemann et al. 2013,145, Olesen et al. 2014,146</td>
<td>Resveratrol</td>
<td>250 mg</td>
<td>27 m</td>
<td>2x/wk HIIT cycling, 1x/wk Crossfit (unspecified)</td>
<td>8 weeks</td>
<td>Smaller increase in VO2max: +12.8 vs. 17.2% ([d= -0.6, [95% CI -2.2, -0.5]]) No difference in time to exhaustion</td>
<td>No difference in PGC-1α mRNA, CYT-C, CS and B-HAD activity</td>
<td>Increased protein carbonyls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polley et al. 2016,194</td>
<td>Resveratrol</td>
<td>500 mg Resveratrol + 10 mg piperine</td>
<td>9 m, 7 f</td>
<td>3x/wk, 30 min forearm wrist flexor exercises</td>
<td>4 weeks</td>
<td>No difference</td>
<td>Increased mitochondrial oxidative capacity</td>
<td>Only placebo increased succinate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Schwarz et al. 2018,157                   | Epicatechin | 200 mg | 20 m, f | 4x/wk cycling, 2x 45-60 min at 50% Wmax, 1x/wk HIIT, 1x/wk SIT | 4 weeks  | Smaller increase in VO2max: +6.1 vs. 22.6% ([d= -0.6, [95% CI -1.5,]

33
<table>
<thead>
<tr>
<th>Animals</th>
<th>Vitamin E, 700 mg/kg</th>
<th>Animals</th>
<th>Vitamin C, 500 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venditti et al. 2014[128]</td>
<td>32 m</td>
<td>Rats</td>
<td>10 weeks</td>
</tr>
<tr>
<td>Asha Devi et al. 2003[134]</td>
<td>46 m</td>
<td>Rats</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Kim et al. 2017[132]</td>
<td>24 m</td>
<td>Rats</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Gomez-Cabrera et al. 2008[118]</td>
<td>24 m</td>
<td>Rats</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Meier et al. 2013[129]</td>
<td>32 m</td>
<td>Mice</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

0.4], no difference in peak aerobic power dehydrogenase activity (+59%)

Increased protein carbonyls Decreased GPx, glutathione reductase

Decreased mitochondrial respiration, NRF-1, NRF-2, PGC-1

Time to exhaustion: +33%

No difference in time to exhaustion

No difference in phosphorylation of p38 MAPK and AMPK, or levels of PGC-1a, NRF-1, TFAM

Time to exhaustion: +27 vs. 187% (d= -19.1, [95% CI -26.8, -11.4]) Reduced expression of NRF-1, TFAM, SOD and GPx

Trend for smaller improvement in VO2max: +4.6 vs. 17.1% (d= -0.9, [95% CI -2.1, 0.3])

No difference in mitochondrial enzymes

No difference in CS Decreased expression of SOD1, PGC-1a, or CD36

No difference in peak power Increased expression of FABP-3

Decreased thiobarbituric acid-reactive substance (TBARS)
<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Baseline</th>
<th>Exercise Protocol</th>
<th>Outcome Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abadi et al. 2013[133]</td>
<td>Vitamin E, Coenzyme Q10, alpha lipoic acid</td>
<td>Standard chow with vitamin E (α-tocopherol, 1000 IU), 0.1% a-lipoic acid, and 0.25% CoQ10</td>
<td>36 m, 36 f</td>
<td>Mice</td>
</tr>
<tr>
<td>Strobel et al. 2011[130]</td>
<td>Vitamin E, alpha lipoic acid</td>
<td>1000 IU vitamin E/kg, 1.6 g/kg ALA</td>
<td>48 m</td>
<td>Rats</td>
</tr>
<tr>
<td>Wadley et al. 2013[159]</td>
<td>Allopurinol</td>
<td>0.25 mg/ml</td>
<td>24 m</td>
<td>Rats</td>
</tr>
<tr>
<td>Lee et al. 2015[158]</td>
<td>Epicatechin</td>
<td>1 mg/kg, twice daily</td>
<td>34 m</td>
<td>Mice</td>
</tr>
<tr>
<td>Dolinsky et al. 2012[195]</td>
<td>Resveratrol</td>
<td>4 g/kg</td>
<td>24 m</td>
<td>Rats</td>
</tr>
<tr>
<td>Hart et al. 2013[149]</td>
<td>Resveratrol</td>
<td>100 mg/kg</td>
<td>24 m</td>
<td>Rats (bred for high endurance capacity)</td>
</tr>
<tr>
<td>Hart et al. 2014[150]</td>
<td>Resveratrol</td>
<td>100 mg/kg</td>
<td>24 m</td>
<td>Rats (bred for low endurance capacity)</td>
</tr>
<tr>
<td>Kan et al. 2016[196]</td>
<td>Resveratrol</td>
<td>25 mg/kg</td>
<td>16 m</td>
<td>Mice</td>
</tr>
<tr>
<td>Murase et al. 2005[155]</td>
<td>Green tea extract</td>
<td>0.2-0.5% (wt/wt)</td>
<td>80 m</td>
<td>Mice</td>
</tr>
<tr>
<td>Murase et al. 2006[152]</td>
<td>Green tea extract</td>
<td>0.2-0.5% (wt/wt)</td>
<td>32 m</td>
<td>Mice</td>
</tr>
</tbody>
</table>

HIIT: High-intensity interval training, HR: Heart rate, SIT: sprint-interval training, TTE: Time to exhaustion, PPO: Peak power output, LT: Lactate threshold, RBI: Rest between intervals
Effect size of supplement calculated as Cohen's d when data were available (reported as effect size, 95% CI)
<table>
<thead>
<tr>
<th>Participants</th>
<th>Training Status</th>
<th>Length</th>
<th>Supplement Dosage</th>
<th>Type of Training</th>
<th>Total Number of Training Sessions</th>
<th>Performance (compared with placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Creatine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graef et al. 2009[187]</td>
<td>33 m</td>
<td>Recreationally Active</td>
<td>4 weeks</td>
<td>10 g per day for the first 10 days, then 10 g per day 5x/wk for three weeks (training days only)</td>
<td>Training 5/wk; 3/wk HIIT, 5x2 min at 90-120% PPO, 1 min RBI; 2x/wk recovery, 5x2 min at 80% PPO, 1 min RBI</td>
<td>20</td>
</tr>
<tr>
<td>Kendall et al. 2009[186]</td>
<td>42 m</td>
<td>Recreationally Active</td>
<td>4 weeks</td>
<td>10 g per day for the first 10 days, then 10 g per day 5x/wk for three weeks (training days only)</td>
<td>Training 5/wk; 3/wk HIIT, 5-6x2 min at 90-120% PPO, 1 min RBI; 2x/wk recovery, 5x2 min at 80% PPO, 1 min RBI</td>
<td>20</td>
</tr>
<tr>
<td>Forbes et al. 2017[188]</td>
<td>17 f</td>
<td>Recreationally Active</td>
<td>4 weeks</td>
<td>0.3 g/kg body mass/ day for 5 days followed by 0.1 g/kg body mass for 23 days</td>
<td>HIIT 3x/wk; 1 session of 4-6 Wingate tests, 4 min RBI; 1 session of 10-20 6 s sprints, 24 s recovery; 1 session of 8-12x 60 sec at 85% and 60 sec at 15% PPO</td>
<td>12</td>
</tr>
</tbody>
</table>

HIIT: High-intensity interval training, TTE: Time to exhaustion, PPO: Peak power output, RBI: Rest between intervals
Effect size of supplement calculated as Cohen’s d when data were available (reported as effect size, 95% CI)
Figure 1. Potential impact of supplements on endurance training adaptations

Green solid line: increases, Red dashed line: inhibits
Figure 2. Schematic of areas where dietary supplements have the potential to impact the adaptive responses to endurance training.

AMP adenosine monophosphate, AMPK 5’ AMP-activated protein kinase, ERK1/2 extracellular-regulated kinase 1 and 2, NADH nicotinamide adenine dinucleotide, p38 MAPK p38 mitogen-activated protein kinase, PGC-1α peroxisome proliferator-activated receptor γ coactivator 1 α, RONS reactive oxygen and nitrogen species, SIRT1 silent mating type information regulation 2 homolog 1. Green solid line: increases, Red dashed line: inhibits. ?= mechanistic potential to occur.
Figure 3. **a** Blood pH responses to high-intensity interval training with ingestion of sodium bicarbonate (Bicarb), ammonium chloride (Acid), or a placebo, **b** effect of pH and training on PGC-1α, and **c** training responses. An alkaline pH can enhance, while a more acidic pH can impair, the acute mRNA response of PGC-1α and longer-term training adaptations to high-intensity interval training. PGC-1α: peroxisome proliferator-activated receptor γ coactivator 1α. *Main effect for time compared with pre-ingestion (P<0.01); †Significant difference from placebo at the time point designated (P<0.01); # Significantly different from placebo and bicarb at the time point designated (p<0.05); ⌟ significant difference from pre-training (p<0.05). Adapted from [22, 27, 33, 35, 37].
Figure 4. Percent improvement in a 4-km time-trial performance and b time to exhaustion after 4-wk of placebo and regular training (Pla), 4-wk of beta-alanine supplementation and regular training (BA), 5-wk of sprint-interval training and placebo (SIT+Pla), 4-wk loading phase of beta-alanine followed by 5-wk of sprint-interval training with continued supplementation (SIT+BA), in trained cyclists. Supplementing with β-alanine while maintaining their normal training (BA) reported a 1.6% improvement in 4-km time-trial performance (~6 min) ($d = 0.3$) and a 7.5% improvement in time-to-exhaustion at 120% of peak power ($d = 0.4$), compared with no changes in the placebo group, while five weeks of SIT with β-alanine resulted in a greater improvement in time-to-exhaustion (+14.9 vs. 9.0%, $d = 0.5$) and a trend for greater improvement in time-trial performance compared with a placebo, suggesting both independent and additive benefits of SIT and β-alanine supplementation. †Significant difference from pre-supplementation ($P < 0.05$), *Significant difference from SIT+Pla. Adapted from [53, 60].
Compliance with Ethical Standards

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**Conflicts of Interest.** Jeffrey Rothschild and David Bishop declare that they have no conflicts of interest relevant to the content of this review.

**References**


