Effects of Vitamin E and Coenzyme Q₁₀ Supplementation on Oxidative Stress Parameters in Untrained Leisure Horses Subjected to Acute Moderate Exercise

Alenka Nemec Svete 1, Tomaž Vovk 2, Mojca Bohar Topolovec 1,3 and Peter Kruljc 3,*

1 Small Animal Clinic, Veterinary Faculty, University of Ljubljana, Gerbičeva ulica 60, 1000 Ljubljana, Slovenia; alenka.nemecsvete@vf.uni-lj.si (A.N.S.)
2 The Chair of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia; tomaz.vovk@ffa.uni-lj.si
3 Clinic for Reproduction and Large Animals, University of Ljubljana, Gerbičeva ulica 60, 1000 Ljubljana, Slovenia
* Correspondence: peter.kruljc@vf.uni-lj.si; Tel.: +386-1-4779-325

Abstract: The effects of antioxidant supplements on exercise-induced oxidative stress have not been investigated in untrained leisure horses. We investigated the effects of 14-day supplementation with vitamin E (1.8 IU/kg/day), coenzyme Q₁₀ (CoQ₁₀; ubiquinone; 800 mg/day), and a combination of both (the same doses as in mono-supplementation) on the blood levels of CoQ₁₀, vitamin E, and oxidative stress parameters in untrained leisure horses subjected to acute moderate exercise. Correlations between lipid peroxidation and muscle enzyme leakage were also determined. Forty client-owned horses were included in the study, with 10 horses in each of the antioxidant and placebo (paraffin oil) groups. Blood parameters were measured before supplementation, before and immediately after exercise, and after 24 h of rest. The differences in individual parameters between blood collection times and groups were analysed with linear mixed models (p < 0.05). None of the supplemented antioxidants affected vitamin E and CoQ₁₀ concentrations, oxidative stress parameters, or serum muscle enzymes. Lipid peroxidation occurred in horses supplemented with placebo and CoQ₁₀ but not in horses supplemented with vitamin E or the combination of both antioxidants. These results suggest that vitamin E alone or in combination with CoQ₁₀ prevented lipid peroxidation in untrained leisure horses subjected to acute moderate exercise.

Keywords: warm-blooded horses; acute moderate exercise; exercise-induced oxidative stress; coenzyme Q₁₀; vitamin E; malondialdehyde; creatine kinase; aspartate aminotransferase; antioxidant enzymes

1. Introduction

The beneficial effects of regular, non-exhaustive physical activity have long been known [1,2]. However, these beneficial effects are lost with strenuous, exhaustive, or unaccustomed exercise, which can lead to muscle damage, inflammation, and oxidative stress [3,4]. In humans and animals, exercise can lead to the increased production of reactive species, reactive nitrogen species (RNS), and especially reactive oxygen species (ROS), which can lead to oxidative stress and consequent oxidative damage to DNA, proteins and lipids, especially in untrained individuals [4–9]. On the other hand, physiological concentrations of ROS play an important role in cell signalling and in regulating gene expression and are thus essential for normal cellular functions [3,4]. In addition, moderate exposure to RNS and ROS is considered necessary for adaptation to exercise training by modulating muscle contraction and the activation of the endogenous antioxidant system, including the increased expression of antioxidant enzymes [4,8–13]. Organisms have evolved remarkably efficient antioxidant defence mechanisms to remove ROS and thereby prevent the damage caused by their action [6,7,14–16]. There
is growing evidence that antioxidant defence systems, including enzymatic and non-enzymatic antioxidants, are capable of major adaptations during acute and chronic exercise [4, 9, 11, 12, 17, 18]. Moderate exercise itself can be considered an antioxidant, as low to moderate concentrations of ROS act as signals that induce the expression of powerful endogenous antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase, etc.) relevant to muscle cell adaptation to exercise as well as other defence mechanisms [3, 4, 11, 18].

Physical activities of different intensities have been shown to induce oxidative stress not only in human athletes but also in horses; namely endurance horses [19–23], show jumpers and dressage horses [24, 25], pentathlon horses [26], Marremmana racehorses [27], Thoroughbred racehorses [28–30], Standardbred trotters [31, 32], and horses subjected to various moderate and intense treadmill exercise tests [33–36]. Lipid peroxidation is the most common consequence of exercise-induced oxidative stress and can lead to the release of muscle enzymes into the systemic circulation. Exercise-induced redox disturbances in skeletal muscle can also contribute to muscle fatigue and muscle injury [8, 16]. In addition, lipid peroxidation induced by physical activity can also lead to myopathy and haemolysis [6, 16, 20, 27, 34, 37–39].

Antioxidant supplements are commonly used to reduce exercise-induced oxidative stress, muscle damage, and inflammation and to enhance exercise performance in human athletes [3, 4, 7, 8, 11, 13, 40–43], as well as in sport horses [44–49]. However, the effects of antioxidant supplements are controversial in exercise research. Antioxidant supplementation can have both beneficial and pro-oxidant effects [4, 7, 44]. In addition, it may hinder or prevent important signalling adaptations to exercise training [12, 13, 50].

Vitamin E (α-tocopherol) is one of the most well-studied and widely used antioxidant supplements in exercise research in humans [8, 13, 41, 50, 51] and horses [34, 45, 47, 49, 52–59]. This exogenous antioxidant is one of the most potent free radical scavengers [60–62]. Vitamin E supplementation has been reported to reduce the concentration of lipid peroxidation products, indicating a protective effect of vitamin E supplementation against oxidative stress induced by various forms of physical activity in humans [63–67] and in sport horses [34, 52]. On the other hand, a meta-analysis based on human studies showed that vitamin E supplementation did not result in significant protection against exercise-induced lipid peroxidation or muscle damage [41]. Similar results have been reported in sport horses [53, 56, 57, 68]. The inconsistency of results regarding the effects of vitamin E supplementation on exercise-induced oxidative stress appears to reflect a variety of factors, including the amount, duration, form, and frequency of vitamin E supplementation; the type and timing of exercise; the age and fitness of the subjects; the vitamin E status of the subjects prior to the studies; and the methodology used to assess oxidative stress [8, 41, 50, 58, 66, 69]. Nevertheless, it should be emphasised that there is limited evidence to support the use of vitamin E in human athletes [70].

Coenzyme Q10 (CoQ10; 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone), also known as ubiquinone, is the only endogenous fat-soluble antioxidant in the organism that is synthesised de novo. Coenzyme Q10 is known for its key role in mitochondrial bioenergetics. In addition, data showed that CoQ10 affects the expression of genes involved in human cell signalling, metabolism, and transport [71]. The reduced form of CoQ10, ubiquinol, is a potent antioxidant and may play a role in recycling tocopherols by effectively reducing the tocopheroxyl radical in mitochondria to alpha-tocopherol, thus regenerating the active form of vitamin E [71–73]. Considering the key role of CoQ10 in cellular energy production, CoQ10 has a potential role in the prevention of exercise-induced oxidative stress [43].

Several studies have reported beneficial, neutral, and adverse effects of CoQ10 supplementation on exercise-induced oxidative stress and exercise capacity [13, 43, 74–76]. These differences could be a consequence of inadequate CoQ10 doses and poor absorption after oral intake. Nevertheless, several studies investigating the effect of CoQ10 supplementation on exercise-induced oxidative stress in humans reported decreased levels of lipid
peroxidation products \[43,75,77–79\]. Furthermore, ubiquinol supplementation minimised the exercise-induced depletion of CoQ\(_{10}\) and increased intracellular and plasma levels of antioxidants; however, it was unable to improve physical performance indices or reduce levels of creatine kinase (CK) and myoglobin, markers of muscle damage \[76\]. The discrepancy and inconsistency of results obtained in studies of CoQ\(_{10}\) supplementation may be due to differences between studies in CoQ\(_{10}\) formulations, dosages, the timing of supplementation, exercise tests performed, types of participants, and outcome measures used \[13,43\].

In contrast to human studies, there are few studies in the literature on CoQ\(_{10}\) supplementation in exercising racehorses \[46,48,80\]. However, there are no reports of CoQ\(_{10}\) as well as vitamin E supplementation in client-owned untrained leisure horses. Therefore, the aim of our study was to investigate the effects of 14 days of supplementation of vitamin E, CoQ\(_{10}\) (ubiquinone) and a combination of both on plasma concentrations of CoQ\(_{10}\) and vitamin E as well as on blood oxidative stress parameters (total antioxidant capacity (TAC), SOD, GPX, and malondialdehyde (MDA)) in untrained leisure horses subjected to acute moderate exercise. In addition, serum muscle enzymes, aspartate aminotransferase (AST) and CK, were determined to investigate possible correlations between lipid peroxidation product, MDA, and serum muscle enzymes.

2. Materials and Methods
2.1. Animals
Sixty-three client-owned, warm-blooded, untrained leisure horses were evaluated for inclusion in our randomised, double-blind, placebo-controlled study. Forty horses of both sexes (18 mares and 22 geldings) and different breeds, with a mean age of 10.6 years (range 3 to 18 years) and a mean weight of 544 kg (range 423 to 697 kg), met the inclusion criteria; young (less than 3 years) and old horses (more than 18 years), mares in foal, mares with foals, and competition horses were not included, as well as horses receiving antioxidant supplements and those with concurrent diseases. Horses were considered healthy based on history, results of physical examination, and results of haematological and biochemical analyses (Supplementary File). The horses were fed oats and hay. Water was provided ad libitum.

Written informed consent was obtained from the owners of the horses before inclusion in the study. All procedures were in compliance with the relevant Slovenian regulations (Animal Protection Act, Official Gazette of the Republic of Slovenia, No. 43/2007). The Ethical Committee of the Ministry of Agriculture, Forestry and Food, Veterinary Administration of the Republic of Slovenia approved all procedures (Licence No. 34401-5/2010/13).

2.2. Testing Protocol
Horses were randomly divided into 4 groups of 10 horses each and received supplements 2 times daily (at 7 a.m. and 19 p.m.) for 14 consecutive days. Horses in the CoQ\(_{10}\) group (G-CoQ\(_{10}\)) received 800 mg/day of CoQ\(_{10}\) (ubiquinone; Q\(_{10}\) Vital Paste 7.5%, Valens Int. d. o. o., Šenčur, Slovenia) in the form of an inclusion complex with β-cyclodextrin (water soluble) produced in accordance with a previously filed patent \[81\]. Horses in the vitamin E group (G-Vit. E) received natural vitamin E (d-α-tocopherol acetate; natural vitamin E-oil, Natural Wealth, Bohemia, NY, USA) in the dose of 1.8 IU/kg BW/day \[82\], and the horses in the CoQ\(_{10}\) + Vit. E group (G-CoQ\(_{10}\) + Vit. E) received 800 mg CoQ\(_{10}\)/day and vitamin E in the same dose as horses in the G-Vit. E group. The dose of vitamin E was calculated based on body weight; adult horses with an average body weight of 550 kg require 990 IU of vitamin E daily during moderate physical activity \[82\]. Horses in the control group (G-placebo) received 2.6 mL/day of paraffin oil (paraffinum liquidum, Ph. Eur. 6.0, series: MH163/10, 1 L, density at 20 °C, 0.865; Pharmachem sp., Ljubljana, Slovenia) as placebo. The calculated dose of paraffin oil was equivalent to a dose of a paste of 800 mg CoQ\(_{10}\)/day.
2.3. Acute Moderate Exercise

All 40 horses performed the acute moderate exercise [82] in the manege (diameter of approximately 18 m) after 14 days of placebo and antioxidant supplementation and were supervised by a licensed trainer. The program included “alternative lunging” in the following order: 10 min walk (warm-up), 5 min slow trot, 10 min fast trot, and 15 min gallop. This was followed by a 15–20 min of cooling down by walk. Respiratory rate, body temperature, and heart rate (Supplementary File) were measured immediately before and immediately after the exercise (40 min after the beginning of the exercise). Environmental factors air temperature and relative humidity were measured before the exercise.

2.4. Sample Collection and Preparation

Blood samples for the determination of haematological (Supplementary File), biochemical (Supplementary File), and oxidative stress parameters (TAC, SOD, GPX, and MDA) and antioxidants (vitamin E and CoQ10) were collected from the jugular vein before the first dose of supplements (basal values—0), after 14 days of supplementation (before exercise—1), at the end of the gallop (immediately after exercise—2), and 24 h after (3) the exercise.

Blood samples for the determination of haematological parameters and MDA were collected into 3 mL tubes containing K3EDTA, for biochemical parameters into 5 mL serum separator tubes and for TAC, SOD, GPX, vitamin E, and CoQ10 into five 2 mL tubes containing lithium heparin (all tubes Vacuette, Greiner, Kremsmünster, Austria). Haematological analysis was performed within 5 h after collection of samples. Samples in serum separator tubes were left to clot for 60 min at room temperature and then centrifuged (1300 × g for 10 min) to separate the serum. All biochemical parameters were determined immediately after serum preparation. Blood samples for the determination of plasma TAC, vitamin E, CoQ10, and MDA were centrifuged at 1500 × g for 15 min at 4 °C. Plasma was separated, immediately frozen, and stored at −80 °C until analysis. Blood samples for the determination of GPX in whole blood lysates and SOD in washed red blood cells were prepared, immediately frozen, and stored at −80 °C until analysis.

2.5. Sample Analysis

Haematological analyses were performed with an Advia 120 automated laser haematology analyser (Siemens, Munich, Germany), biochemical parameters (except electrolytes—sodium, potassium, and chloride) were determined with an RX-Daytona automated biochemical analyser (Randox, Crumlin, England), and concentrations of electrolytes were determined with an Ilyte electrolyte analyser (IL—Instrumentation Laboratory, Lexington, USA). Concentrations of TAC, SOD, and GPX were determined spectrophotometrically with an RX-Daytona automated biochemical analyser (Randox, Crumlin, England) using commercially available reagent kits (total antioxidant status (TAS), Ransod (SOD) and Ransel (GPX); all Randox, Crumlin, England). MDA concentrations were determined by high-performance liquid chromatography (HPLC) and the electrospray ionisation method on an Agilent 6460 triple analyser with a triple quadrupole MS/MS detector (Agilent Technologies, Santa Clara, CA, USA). Plasma samples were treated with a derivatisation method using dinitrophenylhydrazine according to the literature [83]. Total CoQ10 concentrations were determined by HPLC and the atmospheric pressure chemical ionisation method on a Finnigan-LCQ mass spectrometer (Finnigan MAT, San Jose, CA, USA) according to the procedure described previously [84]. Vitamin E concentrations were determined using the HPLC method and an Alliance HPLC System 2695 fluorescence detector (Waters, Milford, MA, USA) according to the procedure described previously [85,86].

2.6. Data Analysis

Data were analysed using software package R (Version 3.0.2 for Windows; R Foundation for Statistical Computing, Vienna, Austria). Differences in individual parameters...
between blood collection times and groups of horses were analysed with linear mixed models [87,88], where fixed factors (fixed effects) take into account the blood collection, group of horses, and their interaction, and each horse was a candidate for the random intersection (random intercept). Multiple comparisons took into account the revised level of features for Bonferroni, $p < 0.002$. R-library was used with nlme mixed models and phia for the post hoc analysis by correcting the Holm–Sidak method. The Pearson correlation coefficient analysis was performed to determine the correlation between MDA and serum muscle enzymes (AST, CK). A value of $p < 0.05$ was considered significant. Data are shown as the means ± standard deviations (SD).

3. Results

No significant changes in plasma vitamin E (Table 1) or CoQ$_{10}$ concentrations (Table 1) were observed in either group of horses between the different sampling times. However, a significantly higher plasma vitamin E concentration was observed in the CoQ$_{10}$ + Vit. E group than in the placebo group at all sampling times. Except at the basal sampling time, plasma CoQ$_{10}$ concentrations were significantly higher in the CoQ$_{10}$ + Vit. E group than in the placebo group at all other sampling times.

Table 1. Plasma vitamin E (µmol/L) and coenzyme Q$_{10}$ (mg/L) concentrations (mean ± SD) in individual groups of horses during the study.

| Group                        | 0         | Blood Sample Collection |
|------------------------------|-----------|-------------------------|
| G-placebo (n = 10)           |           | 1 | 2 | 3 |
| Vitamin E                   | 4.75 ± 1.52 | 4.69 ± 1.39 | 4.71 ± 1.93 | 4.64 ± 1.81 |
| Coenzyme Q$_{10}$           | 2.94 ± 0.82 | 2.48 ± 0.55 | 2.31 ± 0.45 | 2.16 ± 0.43 |
| G-CoQ$_{10}$ (n = 10)        |           |               |            |              |
| Vitamin E                   | 4.77 ± 1.79 | 4.91 ± 1.97 | 4.91 ± 1.89 | 4.82 ± 1.93 |
| Coenzyme Q$_{10}$           | 2.70 ± 0.35 | 2.88 ± 1.18 | 3.10 ± 1.11 | 2.51 ± 0.62 |
| G-Vit. E (n = 10)            |           |               |            |              |
| Vitamin E                   | 3.63 ± 0.79 | 3.87 ± 0.98 | 3.66 ± 0.98 | 3.59 ± 0.96 |
| Coenzyme Q$_{10}$           | 2.47 ± 1.20 | 2.10 ± 0.50 | 2.16 ± 0.43 | 2.21 ± 0.60 |
| G-CoQ$_{10}$ + Vit. E (n = 10)|           |               |            |              |
| Vitamin E                   | 7.73 ± 2.87 * | 8.43 ± 3.41 * | 8.19 ± 3.38 * | 8.18 ± 3.43 * |
| Coenzyme Q$_{10}$           | 3.17 ± 0.88 | 5.04 ± 1.92 * | 4.07 ± 2.36 * | 4.49 ± 0.69 * |

* $p < 0.05$ compared to the placebo group. 0, before adding antioxidant supplements; 1, before acute moderate exercise; 2, immediately after acute moderate exercise; 3, 24 h after acute moderate exercise; G-placebo, control group of horses supplemented with placebo; G-CoQ$_{10}$, group of horses supplemented with CoQ$_{10}$; G-Vit. E, group of horses supplemented with vitamin E; G-CoQ$_{10}$ + Vit. E, group of horses supplemented with combination of CoQ$_{10}$ and vitamin E; n, number of horses included in the group.

Before supplementation, MDA concentrations (Table 2) did not differ significantly between horse groups. MDA concentrations increased significantly after exercise compared with concentrations before exercise in the placebo group and in the group of horses supplemented with CoQ$_{10}$ (Table 2). In the placebo group, MDA concentrations remained significantly elevated even after 24 h of rest. However, no significant difference in MDA concentration was found between the placebo group and each group of horses supplemented with antioxidants at any of the sampling time points. Furthermore, MDA concentration was significantly higher in the CoQ$_{10}$ group compared to the CoQ$_{10}$ + Vit. E group after exercise.
Table 2. The MDA concentrations (µmol/L) (mean ± SD) in individual groups of horses during the study.

| Group                  | 0                | 1                | 2                | 3                |
|------------------------|------------------|------------------|------------------|------------------|
| G-placebo              | 4.99 ± 0.88      | 4.26 ± 0.95      | 6.29 ± 2.33 a    | 6.34 ± 2.06 a    |
| G-CoQ10                | 5.66 ± 0.90      | 5.98 ± 1.15      | 7.55 ± 2.31 a,** | 6.33 ± 1.81      |
| G-Vit. E               | 5.16 ± 0.60      | 5.71 ± 1.37      | 5.53 ± 1.68      | 5.34 ± 1.37      |
| G-CoQ10 + Vit. E       | 4.94 ± 1.17      | 6.17 ± 2.05      | 5.13 ± 0.87      | 5.21 ± 1.67      |

a p < 0.05 compared to blood collection time 1 (within group comparison); ** p < 0.05 compared to the G-CoQ10 + Vit. E group. 0, before adding antioxidant supplements; 1, before acute moderate exercise; 2, immediately after acute moderate exercise; 3, 24 h after acute moderate exercise; G-placebo, control group of horses supplemented with placebo; G-CoQ10, group of horses supplemented with CoQ10; G-Vit. E, group of horses supplemented with vitamin E; G-CoQ10 + Vit. E, group of horses supplemented with combination of CoQ10 and vitamin E; MDA, malondialdehyde.

Before supplementation, the TAC concentrations (Table 3) and activity of SOD (Table 4) did not differ significantly between the horse groups. During the study, the TAC levels (Table 3) and activity of SOD (Table 4) did not change significantly between the different sampling times in either group of horses. Moreover, we did not find significant differences in TAC values and SOD activities between the horse groups at any of the sampling times. On the other hand, the activity of GPX (Table 4) showed significant differences between the horse groups before supplementation. Compared to the placebo group, GPX activities were significantly high in horses supplemented with vitamin E and in horses supplemented with a combination of vitamin E and CoQ10 at all sampling time points. In horses supplemented with the combination of vitamin E and CoQ10, GPX activities were significantly increased after 24 h of rest compared to pre- and post-exercise activities.

Table 3. The TAC concentrations (mmol/L) (mean ± SD) in individual groups of horses during the study.

| Group               | 0                | 1                | 2                | 3                |
|---------------------|------------------|------------------|------------------|------------------|
| G-placebo           | 1.22 ± 0.09      | 1.22 ± 0.07      | 1.26 ± 0.77      | 1.21 ± 0.05      |
| G-CoQ10             | 1.22 ± 0.08      | 1.27 ± 0.12      | 1.28 ± 0.09      | 1.22 ± 0.09      |
| G-Vit. E            | 1.14 ± 0.08      | 1.17 ± 0.08      | 1.18 ± 0.11      | 1.11 ± 0.08 b    |
| G-CoQ10 + Vit. E    | 1.16 ± 0.09 b    | 1.21 ± 0.06      | 1.23 ± 0.05      | 1.19 ± 0.08 b    |

b p < 0.05 compared to blood collection time 2 (within group comparison). 0, before adding antioxidant supplements; 1, before acute moderate exercise; 2, immediately after acute moderate exercise; 3, 24 h after acute moderate exercise; G-placebo, control group of horses supplemented with placebo; G-CoQ10, group of horses supplemented with CoQ10; G-Vit. E, group of horses supplemented with vitamin E; G-CoQ10 + Vit. E, group of horses supplemented with combination of CoQ10 and vitamin E; TAC, total antioxidant capacity.

Before supplementation, the activities of CK and AST (Table 5) did not differ significantly between the horse groups. In addition, we did not find significant differences in the activity of these two enzymes (Table 5) between the horse groups at any of the other sampling times. During the study, the activity of CK did not change significantly in either group of horses at the different sampling times. Regarding AST activity, we found a significant difference between sampling times 2 and 3 in the group supplemented with CoQ10 (Table 5). In the other groups of horses, no significant differences in AST activity were found between the different sampling times.
Table 4. The SOD (U/g Hb) and GPX activities (U/g Hb) (mean ± SD) in individual groups of horses during the study.

| Group          | Blood Sample Collection |
|----------------|-------------------------|
|                | 0 | 1 | 2 | 3 |
| G-placebo      |   |   |   |   |
| SOD            | 1809.8 ± 415.4 | 1814.5 ± 263.7 | 1707.1 ± 336.3 | 1710.9 ± 384.2 |
| GPX            | 75.2 ± 30.2    | 76.0 ± 29.5    | 81.8 ± 33.1    | 83.7 ± 34.5    |
| G-CoQ10        |   |   |   |   |
| SOD            | 1870.4 ± 360.9 | 1894.5 ± 287.7 | 1882.3 ± 328.8 | 1915.7 ± 378.4 |
| GPX            | 56.0 ± 23.6 ** | 60.7 ± 24.4 ** | 63.1 ± 26.4 ** | 62.2 ± 25.3 ** |
| G-Vit. E       |   |   |   |   |
| SOD            | 1932.6 ± 234.9 | 1915.6 ± 175.9 | 1869.7 ± 200.2 | 1897.0 ± 169.1 |
| GPX            | 204.6 ± 19.7 ***| 207.6 ± 21.6 ***| 212.2 ± 24.0 ***| 214.2 ± 20.2 * |
| G-CoQ10 + Vit. E|   |   |   |   |
| SOD            | 1898.0 ± 291.4 | 1906.7 ± 290.6 | 1999.8 ± 362.4 | 1932.4 ± 337.1 |
| GPX            | 126.4 ± 44.1 *  | 126.8 ± 42.8 *  | 126.3 ± 41.6 *  | 198.3 ± 17.4 ** |

* p < 0.05 compared to blood collection time 1 (within group comparison); ** p < 0.05 compared to blood collection time 2 (within group comparison); *** p < 0.05 compared to the placebo group; a,b p < 0.05 compared to the CoQ10 + Vit. E group; 0, before adding antioxidant supplements; 1, before acute moderate exercise; 2, immediately after acute moderate exercise; 3, 24 h after acute moderate exercise; G-placebo, control group of horses supplemented with placebo; G-CoQ10, group of horses supplemented with CoQ10; G-Vit. E, group of horses supplemented with vitamin E; G-CoQ10 + Vit. E, group of horses supplemented with combination of CoQ10 and vitamin E; SOD, superoxide dismutase; GPX, glutathione peroxidase.

Table 5. The CK (U/L) and AST (U/L) activities (mean ± SD) in individual groups of horses during the study.

| Group         | Blood Sample Collection |
|---------------|-------------------------|
|               | 0 | 1 | 2 | 3 |
| G-placebo     |   |   |   |   |
| CK            | 181.0 ± 34.9 | 180.3 ± 26.3 | 218.2 ± 34.1 | 204.7 ± 35.3 |
| AST           | 297.3 ± 50.5 | 296.1 ± 47.0 | 314.3 ± 43.8 | 295.2 ± 40.8 |
| G-CoQ10       |   |   |   |   |
| CK            | 295.6 ± 113.5 | 284.5 ± 110.3 | 304.2 ± 115.0 | 310.7 ± 126.6 |
| AST           | 350.1 ± 76.7 | 356.6 ± 88.7 | 372.2 ± 89.1 | 346.1 ± 76.8 b |
| G-Vit. E      |   |   |   |   |
| CK            | 225.8 ± 100.2 | 242.9 ± 77.5 | 267.7 ± 90.4 | 256.4 ± 91.4 |
| AST           | 328.3 ± 87.6 | 327.4 ± 76.1 | 331.6 ± 74.6 | 326.3 ± 71.3 |
| G-CoQ10 + Vit. E|   |   |   |   |
| CK            | 184.6 ± 51.6 | 271.7 ± 121.3 | 391.2 ± 175.3 | 304.3 ± 167.4 |
| AST           | 293.8 ± 65.1 | 309.8 ± 80.1 | 328.2 ± 89.9 | 317.5 ± 125.3 |

b p < 0.05 compared to blood collection time 2 (within group comparison); 0, before adding antioxidant supplements; 1, before acute moderate exercise; 2, immediately after acute moderate exercise; G-placebo, control group of horses supplemented with placebo; G-CoQ10, group of horses supplemented with CoQ10; G-Vit. E, group of horses supplemented with vitamin E; G-CoQ10 + Vit. E, group of horses supplemented with combination of CoQ10 and vitamin E; CK, creatine kinase; AST, aspartate aminotransferase.

The results of statistical analyses showed no significant correlations between MDA and any of the serum muscle enzymes (AST, CK) in either group of horses at any of the sampling time points.

4. Discussion

To the authors’ knowledge, the present study is the first to report the effects of 14 days of supplementation with vitamin E, CoQ10, and a combination of both antioxidants on the plasma levels of CoQ10 and vitamin E and on blood oxidative stress parameters in untrained leisure horses subjected to acute moderate exercise. Contrary to our expectations, the supplementation of horses with vitamin E or CoQ10 did not result in increased plasma...
concentrations of vitamin E or CoQ\textsubscript{10}, respectively. However, supplementation with a combination of both antioxidants significantly increased plasma CoQ\textsubscript{10} but not vitamin E concentrations. Although vitamin E and CoQ\textsubscript{10} are known for their antioxidant properties and the sparing effect of CoQ\textsubscript{10} on vitamin E, neither the individually supplemented antioxidants nor the co-supplementation of both affected oxidative stress parameters at any of the sampling time points.

In our study, untrained leisure horses were supplemented with 1.8 IU/kg/day of vitamin E as recommended for moderate physical activity for adult horses [82]. The dose of vitamin E used in our study was lower compared to vitamin E supplementation studies in sport horses. The doses used in these studies vary widely. In Thoroughbred and Quarter horses, vitamin E doses were 80 IU/kg dry matter (DM) and 300 IU/kg DM, respectively [53]; in sport horses, about 9 IU/kg/day (3 g alpha-tocopherol/day) [56]; in Standardbred horses, 10–20 IU/kg/day [57]; in Thoroughbred horses, 6 IU/kg/day [68]; and in exercising horses (trotters and riding horses) 1.5, 4.5, and 7.5 IU/kg/day (1, 3, and 5 mg of vitamin E/kg/day) [53,56,59]. Despite supplementation with higher doses of vitamin E for a longer period of time than in our study, the serum or plasma concentrations of vitamin E did not increase [53,56,57,68], which is in agreement with our results [53,56,57,68]. Moreover, serum or plasma concentrations of vitamin E decreased significantly after the supplementation period in exercising sport horses [53,56]. On the other hand, Saastamoinen and Juusela [59] reported a significant increase in serum vitamin E concentration after the long-term supplementation of sport horses with 3 and 5 mg/kg/day of vitamin E, but not after supplementation with 1 mg/kg/day. These authors suggested that sport horses in training require a daily supplement of 3 or 5 mg vitamin E/kg/day to increase the serum vitamin E concentrations. The absorption of vitamin E requires the presence of fat [62,89,90]. Therefore, a lack of fat in the diet of horses can lead to the poor absorption of vitamin E [57,90]. Not only the presence of adequate amounts of fats in the diet but also the dietary matrix itself and the concentration of lipoproteins have an influence on the absorption of vitamin E [62,89,90]. In contrast to our results, some studies conducted in sport horses showed a significant increase in plasma or serum vitamin E concentrations after vitamin E supplementation [49,51,52,58,59]. Untrained leisure horses included in our study are a much more diverse group of horses (different breeds, differences in level and frequency of physical activity, and different diets and environmental factors) compared to groups of sport horses. Therefore, differences between studies could be due to differences in the horses included in these studies, as well as differences in diets, the duration of supplementation, doses, and forms of vitamin E. The form and source of vitamin E supplementation is very important in horses because it significantly affects not only plasma vitamin E concentrations [91] but also the level of oxidative stress parameters in blood samples [49]. Serum vitamin E concentration significantly increased after 14 days of supplementation with synthetic vitamin E and natural source of vitamin E (micellized form of vitamin E; RRR \( \alpha \)-tocopherol) at the dose of 4000 IU/day, and the micellized form of vitamin E was superior to the synthetic form. On the other hand, no significant increase in serum vitamin E concentration was observed in the control group supplemented with a similar dose of vitamin E (1000 IU/day) as in our study [49]. Similarly, Pagan and colleagues [91] reported that supplementation with the micellized form of vitamin E in Thoroughbred horses was superior in increasing plasma vitamin E concentrations compared to synthetic vitamin E (dl-alpha-tocopheryl acetate) and a natural source of vitamin E (d-alpha-tocopheryl acetate) [91]. The latter was used in our study. In some exercise studies in humans, vitamin E supplementation increased plasma vitamin E concentrations [50,65,92,93], whereas in many other studies, plasma vitamin E concentrations were not measured [8,92].

Overall, the lack of a significant increase in plasma vitamin E concentration in our study could be due to the inappropriate form of this antioxidant supplement, inadequate dose and short duration of supplementation, individual differences in leisure horses, and poor absorption after oral intake.
Similar to vitamin E, CoQ₁₀ supplementation did not significantly increase plasma CoQ₁₀ concentrations in our leisure horses. In Thoroughbred racehorses, significantly increased serum CoQ₁₀ concentrations were found at days 30 and 60 of CoQ₁₀ supplementation with the same CoQ₁₀ dose as that used in our study [80]. The difference between the studies could be due to a longer period of supplementation [80], the use of different CoQ₁₀ formulations, and differences in the horses included in the studies. Increased plasma CoQ₁₀ concentrations were obtained after the exercise and supplementation of Thoroughbred racehorses with much higher daily doses of CoQ₁₀, 1.9 g and 3.4 g, respectively [46]. Interestingly, in Thoroughbred racehorses, Thueson and colleagues reported significantly higher CoQ₁₀ concentrations in skeletal muscle after 10 days of supplementation with 1000 mg of ubiquinol [48]. In humans, CoQ₁₀ supplementation leads to an increase in plasma CoQ₁₀ concentrations, the magnitude of which depends on the dosage, duration, and also the type of formulation [94–96]. Most studies in human athletes have found increased serum or plasma CoQ₁₀ concentrations following supplementation with ubiquinone or ubiquinol [43,75–79,97,98]. The lack of a significant increase in plasma CoQ₁₀ concentration after supplementation with this antioxidant in our study could be due to the short duration of supplementation, inadequate dose of CoQ₁₀, and poor absorption. Being a lipophilic substance, the absorption of CoQ₁₀ follows the same process as that of lipids in the gastrointestinal tract. The absorption mechanism for CoQ₁₀ appears to be similar to that of vitamin E. Similar to vitamin E, the absorption of CoQ₁₀ is enhanced in the presence of lipids. Therefore, the absorption of supplemental CoQ₁₀ may be increased when taken with fatty meals, which was not the case in the group of leisure horses studied. Furthermore, in humans, plasma CoQ₁₀ concentrations are highly dependent on plasma lipoproteins [94,99]. We may assume that the lack of fat in horse meal, as well as the low concentrations of lipoproteins in horse blood [100], could also lead to poor absorption and thus the lack of a significant increase in plasma CoQ₁₀ after supplementation. In contrast to humans, high-density lipoproteins are the major lipoprotein fraction in horses, followed by low-density lipoproteins; the smallest proportion of lipoproteins belongs to the very-low-density lipoproteins [100].

Interestingly, the supplementation of leisure horses with a combination of vitamin E and CoQ₁₀ resulted in a significant increase in plasma CoQ₁₀ but not vitamin E concentrations. Our results are in partial agreement with the results of a double-blind, placebo-controlled study in marathon runners [101], in which co-supplementation with these two antioxidants resulted in a significant increase in plasma concentrations of both antioxidants. Zhou and colleagues also reported increased CoQ₁₀ concentrations pre- and post-exercise in men following supplementation of CoQ₁₀ and the combination of CoQ₁₀ and vitamin E; however, the plasma vitamin E concentrations were not measured in their study [102]. In human blood, 95% of CoQ₁₀ is present in its reduced form, as ubiquinol, a potent lipophilic antioxidant [94,103]. The latter is capable of regenerating vitamin E from tocopheroxyl radicals in mitochondria, resulting in sparing of vitamin E and consumption of ubiquinol [104,105]. Therefore, supplementation with a combination of these two antioxidants would also be expected to result in a significant increase in plasma vitamin E concentration [106]. Vitamin E and CoQ₁₀ are both lipophilic antioxidants with similar absorption and transport mechanisms, which could influence the concentration of both antioxidants in plasma. We hypothesise that increased plasma CoQ₁₀ concentration observed in our horses after supplementation with CoQ₁₀ + Vit. E could be due to the antioxidant properties of vitamin E or a synergistic effect of co-supplementation [107].

Numerous studies in humans and horses have investigated the effects of antioxidant supplementation on exercise-induced oxidative stress with equivocal results. This is most likely due to the wide variety of exercise and/or supplementation protocols, including the length of the supplementation period and form of antioxidants and exercise selected by the research group, as well as differences in the fitness of the athletes and sport horses included in the studies [8,13,41–44,50,74]. According to the results of the statistical comparison of oxidative stress parameters between the supplemented groups of leisure horses and the
placebo group, antioxidant supplementation had no effect on oxidative stress parameters at any of the measurement time points. However, a significant increase in MDA concentration after the acute moderate exercise in the placebo and CoQ10 groups but not in vitamin E and CoQ10 + Vit. E groups suggests that vitamin E alone or in combination with CoQ10 prevented lipid peroxidation.

Malondialdehyde is a reliable and the most commonly used marker for the overall lipid peroxidation level and thus for the presence of oxidative stress [108,109]. It is the most commonly used marker of the level of lipid peroxidation in exercise and sport studies [110]. Several studies have confirmed the increased lipid peroxidation during different forms of physical activity in sport horses [19,21,23,24,27,31,32,34,111]. Similarly, in the present study, plasma MDA concentration increased significantly after physical activity in both the placebo and CoQ10 supplemented groups, while no significant increase was observed in the vitamin E and CoQ10 + Vit. E groups. In the placebo group, plasma MDA concentration remained significantly elevated even after 24 h of rest, indicating a higher extent of lipid peroxidation than in the CoQ10 group, although we found no significant difference in MDA concentration between the placebo and CoQ10 groups. These results may suggest that vitamin E and the combination of vitamin E and CoQ10 decreased the process of lipid peroxidation during exercise due to the antioxidant properties of vitamin E. Our findings are in agreement with the results of some studies that investigated the effects of vitamin E supplementation on exercise-induced oxidative stress in sport horses [31,57,68] and in humans [41,67]. The results of the meta-analysis showed that tocopherol supplementation did not result in significant protection against either exercise-induced lipid peroxidation or muscle damage. The lack of protective effects in general was explained by the complex behaviour of tocopherols, which can act as antioxidants, pro-oxidants, and neutral agents in vivo, and (or) possibly by insufficient accumulation in muscle tissue after supplementation [41]. Pro-oxidant properties have been demonstrated in highly trained human athletes, in which vitamin E supplementation resulted in an even greater increase in lipid peroxidation products than placebo supplementation after exhaustive exercise [93]. On the other hand, several studies in human athletes [63–65,67,92] and sport horses [34,52,58] have shown decreased levels of lipid peroxidation products following vitamin E supplementation, suggesting an antioxidant effect of vitamin E.

In contrast to our results, in most human studies, CoQ10 supplementation significantly reduced the concentration of lipid peroxidation products after different types of physical activities [43,75–79,112], indicating the antioxidant effect of CoQ10. In these studies, CoQ10 supplementation resulted in a significant increase in plasma CoQ10 concentration, which was not the case in our study.

The total antioxidant capacity is a biochemical parameter suitable for assessing the overall antioxidant status of serum or plasma resulting from the uptake and/or production of antioxidants and their consumption during normal or increased oxidative stress. The capacity of known and unknown antioxidants and their synergistic interaction are assessed, thus providing insight into the delicate balance between oxidants and antioxidants in vivo [113,114]. Supplementation with antioxidants can increase plasma TAC concentrations [114–116]; however, in our study, TAC concentrations remained unaffected by supplementation with any of the antioxidants at any of the sampling time points. Similar results were obtained by Duberstein and colleagues, who investigated the effect of training and vitamin E supplementation on oxidative stress parameters in exercising horses and used the same method for TAC determination [68], as well as in several exercise studies in human athletes supplemented with vitamin E [67], CoQ10 [77], and a combination of vitamin E and CoQ10 [101]. In contrast to our results, some human studies found increased TAC levels after supplementation with CoQ10 and strenuous exercise [75,79]. The lack of a significant increase in TAC levels after supplementation in our study could simply be due to the fact that there was no significant increase in plasma vitamin E and/or CoQ10 concentrations after supplementation or in the species used in our study. The latter could differ in the metabolism of the supplemented antioxidants compared to humans. In addi-
tion, the lack of a significant increase in plasma TAC concentrations could be partly due to methodological reasons. The methods used for TAC determination vary greatly, meaning that the results of different methods are not comparable [113,114,117].

In our study, TAC values did not show significant changes between different sampling times in any group of horses. Similarly, Balogh and colleagues found no significant differences in TAC levels before or after exercise in pentathlon horses, using the same method for TAC determination as in our study. However, in this study [26], TAC values measured by the method FRAP (ferric reducing ability of plasma) were significantly higher immediately after exercise than before exercise, supporting the idea of measuring TAC with two different methods rather than one. Our findings are similar to the results of studies conducted in sport horses [35,118], in which the chosen physical activity was not intense enough to cause the release of antioxidants that contribute to the increase in plasma or serum TAC concentrations [18]. On the other hand, Duberstein and colleagues reported significantly lower TAC levels after a standard exercise test in placebo and vitamin E supplemented horses [68]. Levels returned to baseline by 48 h after exercise. In addition, Duberstein and colleagues showed increased TAC concentrations in placebo and vitamin E-supplemented horses after five weeks of training, indicating that fitness must be considered when comparing TAC concentrations in exercising horses [68]. Significantly lower TAC levels were also reported in endurance horses at the midpoint (80 km) of the 160 km ride [19]. During and immediately after exercise, TAC may be temporarily lowered, as its components are used to quench the ROS produced. Later, during recovery, antioxidants are released from adipose tissue and the liver, and TAC concentrations usually rise above basal levels. As with other markers, studies reporting no change in antioxidant capacity after exercise, as was the case in our study, may have missed such changes because only one sample was taken immediately after exercise [7].

It is generally agreed that regular endurance training promotes the upregulation of antioxidant genes, leading to an increase in both total SOD and GPX activity in active skeletal muscle. Furthermore, high-intensity exercise training is superior to low-intensity exercise training in terms of the upregulation of muscle SOD and GPX activities [3,4,12,13,18]. Specific antioxidant enzyme activity has been shown to respond similarly to the TAC response to exercise. The antioxidant defence system may be temporarily reduced in response to increased ROS production but may increase again during the recovery period as a result of the initial pro-oxidant insult [7].

In horses, changes in the activity of the primary antioxidant enzymes, SOD and GPX, in response to exercise vary in the literature. In sport horses, significantly higher [19,20,29,57,119,120] or significantly lower activities [24,28,111] of blood antioxidant enzymes, either SOD and/or GPX, have been found after exercise as a result of increased ROS production, either by the upregulation of enzyme activity or by the increased utilisation of antioxidant enzymes to counteract ROS [3,9,13,18]. Moreover, no significant changes in antioxidant enzyme activities have been reported in sport horses [26,28,35,36,121,122]. Similarly, our study showed no significant changes in SOD and GPX activities post-exercise in comparison with pre-exercise values in any of the horse groups. It seems that in our study, the acute moderate exercise was not intense enough to induce changes in the activity of GPX and SOD. Conflicting results regarding changes in blood SOD and GPX activities could reflect differences in the intensity, duration, and type of exercise or training, as well as being due to differences in the timing of sampling and large individual differences between horses participating in the studies [6,7,44]. Similar conflicting results have been reported in exercise research in humans [7,69].

In our study, we found significantly higher GPX activity in the groups supplemented with vitamin E and with the combination of both antioxidants compared with the placebo group at all the measurement time points, which could be due to large interindividual differences in the activity of this antioxidant enzyme in our leisure horses. Nevertheless, we observed no significant effect of antioxidant supplementation on SOD and GPX activities.
Similar results were found in sport horses after supplementation with CoQ_{10} (unpublished data) and vitamin E [28,52,57] and in humans after supplementation with CoQ_{10} [75,79].

Associations between exercise-induced oxidative stress and muscle enzymes leakage have been previously confirmed in sport horses [19,20,54,119]. Our study included untrained leisure horses, so we expected an increase in the activity of the serum muscle enzymes CK and AST, indicating some degree of muscle damage. However, we did not find a significant difference in the activities of AST and CK between pre- and post-exercise sampling times in any of the horse groups, suggesting that our leisure horses respond to acute moderate exercise without the presence of muscle damage that would lead to increased leakage of muscle enzymes into the circulation.

Furthermore, the activities of AST and CK were not affected by any of the antioxidant supplements. In addition, we did not find significant correlations between the lipid peroxidation marker MDA and serum muscle enzymes in any of the groups of leisure horses. A meta-analysis showed no significant effect of vitamin E supplementation on muscle damage in human athletes [8,41]. On the other hand, Helgheim and colleagues reported no significant increases in serum muscle enzyme activity in trained individuals after heavy exercise and a significant increase in CK in the untrained muscle group. Vitamin E supplementation had no effect on serum muscle enzymes after exercise in the trained muscle group [123]. Similar to our results, CoQ_{10} supplementation had no effect on CK activity in moderately trained healthy men [74].

5. Conclusions

Contrary to our expectations, supplementation with vitamin E and CoQ_{10} did not increase the plasma concentrations of these two antioxidants. However, supplementation with a combination of CoQ_{10} and vitamin E increased plasma CoQ_{10} concentrations compared with those of the placebo group. As indicated by a significant increase in MDA concentrations after acute moderate exercise, lipid peroxidation occurred in horses supplemented with placebo and CoQ_{10} but not in horses supplemented with vitamin E or the combination of vitamin E and CoQ_{10}. These results suggest that vitamin E alone or in combination with CoQ_{10} prevented lipid peroxidation. However, we did not find a correlation between lipid peroxidation and serum muscle enzymes. Our results warrant further studies in larger groups of leisure horses using different dosing regimens and antioxidant formulations to determine the effect of antioxidant supplementation on exercise-induced oxidative stress.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antiox10060908/s1. Supplementary file containing all results presented in the manuscript as well as results of haematological and biochemical analyses, body temperature, respiration, and heart rate in individual horses at different sampling time points.

Author Contributions: Conceptualisation, P.K. and A.N.S.; methodology, A.N.S., M.B.T., T.V. and P.K.; formal analysis, A.N.S., T.V. and P.K.; investigation, A.N.S., M.B.T. and P.K.; data curation, A.N.S., M.B.T., T.V. and P.K.; writing—original draft preparation, A.N.S. and M.B.T.; writing—review and editing, A.N.S., T.V. and P.K.; supervision, P.K. With the exception of M.B.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Slovenian Research Agency (ARRS), Grant No. P4-0053 and P1-0189. The APC was funded by the Slovenian Research Agency, Grant No. P4 0053. The funders were not involved in the study design; collection, analyses, and interpretation of data; or writing of the manuscript.

Institutional Review Board Statement: All procedures complied with the relevant Slovenian regulations (Animal Protection Act, Official Gazette of the Republic of Slovenia, No. 43/2007). The Ethical Committee of the Ministry of Agriculture, Forestry and Food, Veterinary Administration of the Republic of Slovenia approved all procedures (Licence No. 34401-5/2010/13).

Informed Consent Statement: Written informed consent was obtained from the owners of horses before inclusion in the study.
Data Availability Statement: The data from this work are included in this article and in the Supplementary Materials and may be obtained from the corresponding author on reasonable request.

Acknowledgments: The authors thank Mateja Blas, statistician, for performing the statistical analysis, and Aleksander Jenko for technical assistance in processing the blood samples.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Warburton, D.E.; Nicol, C.W.; Bredin, S.S. Health benefits of physical activity: The evidence. CMAJ 2006, 174, 801–809. [CrossRef] [PubMed]
2. Siddiqui, N.I.; Nessa, A.; Hossain, M.A. Regular physical exercise: Way to healthy life. Mymensingh Med. J. 2010, 19, 154–158. [PubMed]
3. Gomez-Cabrera, M.C.; Domenech, E.; Vina, J. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. Free Radic. Biol. Med. 2008, 44, 126–131. [CrossRef] [PubMed]
4. He, F.; Li, J.; Liu, Z.; Chuang, C.C.; Yang, W.; Zuo, L. Redox Mechanism of Reactive Oxygen Species in Exercise. Front. Physiol. 2016, 7, 486. [CrossRef]
5. Powers, S.K.; Nelson, W.B.; Hudson, M.B. Exercise-induced oxidative stress in humans: Cause and consequences. Free Radic. Biol. Med. 2011, 51, 942–950. [CrossRef]
6. Deaton, C.M.; Marlin, D.J. Exercise-associated oxidative stress. Clin. Tech. Equine Pract. 2003, 2, 278–291. [CrossRef]
7. Fisher-Wellman, K.; Bloomer, R.J. Acute exercise and oxidative stress: A 30 year history. Dyn. Med. 2009, 8, 1. [CrossRef]
8. Martinez-Ferran, M.; Sanchis-Gomar, F.; Lavie, C.J.; Lippi, G.; Pareja-Galeano, H. Do Antioxidant Vitamins Prevent Exercise-Induced Muscle Damage? A Systematic Review. Antioxidants 2020, 9, 372. [CrossRef]
9. Powers, S.K.; Talbert, E.E.; Adhihetty, P.J. Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle. J. Physiol. 2011, 589, 2129–2138. [CrossRef]
10. Westerblad, H.; Allen, D.G. Emerging roles of ROS/RNS in muscle function and fatigue. Antioxid. Redox Signal. 2011, 15, 2487–2499. [CrossRef]
11. Yavari, A.; Javadi, M.; Mirmiran, P.; Bahadoran, Z. Exercise-induced oxidative stress and dietary antioxidants. Asian J. Sports Med. 2015, 6, e24898. [CrossRef]
12. Merry, T.L.; Ristow, M. Do antioxidant supplements interfere with skeletal muscle adaptation to exercise training? J. Physiol. 2016, 594, 5135–5147. [CrossRef]
13. Mason, S.A.; Trewin, A.J.; Parker, L.; Wadley, G.D. Antioxidant supplements and endurance exercise: Current evidence and mechanistic insights. Redox Biol. 2020, 35, 101471. [CrossRef]
14. Soffler, C. Oxidative stress. Vet. Clin. N. Am. Equine Pract. 2007, 23, 135–157. [CrossRef]
15. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M.T.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 2007, 39, 44–84. [CrossRef]
16. Powers, S.K.; Jackson, M.J. Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. Physiol. Rev. 2008, 88, 1243–1276. [CrossRef]
17. Powers, S.K.; Lennon, S.L. Analysis of cellular responses to free radicals: Focus on exercise and skeletal muscle. Proc. Nutr. Soc. 1999, 58, 1025–1033. [CrossRef]
18. Ji, L.L. Modulation of skeletal muscle antioxidant defense by exercise: Role of redox signaling. Free Radic. Biol. Med. 2008, 44, 142–152. [CrossRef]
19. Frankiewicz-Jozko, A.; Szarska, E. Anti-oxidant level to exercise in the blood of endurance horses. Biol. Sport 2000, 17, 217–227.
20. Hargreaves, B.J.; Kronfeld, D.S.; Waldron, J.N.; Lopes, M.A.; Gay, L.S.; Saker, K.E.; Cooper, W.L.; Sklan, D.J.; Harris, P.A. Antioxidant status and muscle cell leakage during endurance exercise. Equine Vet. J. Suppl. 2002, 116–121. [CrossRef]
21. Marlin, D.J.; Fenn, K.; Smith, N.; Deaton, C.D.; Roberts, C.A.; Harris, P.A.; Dunster, C.; Kelly, F.J. Changes in circulatory antioxidant status in horses during prolonged exercise. J. Nutr. 2002, 132, 1622S–1627S. [CrossRef] [PubMed]
22. Williams, C.A.; Kronfeld, D.S.; Hess, T.M.; Waldron, J.N.; Saker, K.E.; Hoffman, R.M.; Harris, P.A. Oxidative stress in horses in three 80-km races. Equine Physiol. Phys. Soc. Proc. 2003, 18, 47–52.
23. Bottegaro, N.B.; Gotic, J.; Suran, J.; Brozic, D.; Klobucar, K.; Bojanic, K.; Urbanc, Z. Effect of prolonged submaximal exercise on serum oxidative stress biomarkers (d-ROMs, MDA, BAP) and oxidative stress index in endurance horses. BMC Vet. Res. 2018, 14, 216.
24. Muñoz-Escass, B.; Marañón, G.; Manley, W.; de la Muela, M.S.; Riber, C.; Cayado, P.; León, R.; García, C.; Suárez, M.; Vara, E. Exercise-Induced Changes on Lipid Peroxides and Antioxidant Enzymes Levels Changes in Plasma of Show Jumping and Dressage Horses. Intern. J. Appl. Res. Vet. Med. 2006, 4, 274–282.
25. Soares, J.C.M.; Zanella, R.; Bondan, C.; Alves, L.P.; de Lima, M.R.; da Motta, A.C.; Zanella, E.L. Biochemical and Antioxidant Changes in Plasma, Serum, and Erythrocytes of Horses before and after a Jumping Competition. J. Equine Vet. Sci. 2011, 31, 357–360. [CrossRef]
26. Balogh, N.; Gaal, T.; Ribiczeyne, P.S.; Petri, A. Biochemical and antioxidant changes in plasma and erythrocytes of pentathlon horses before and after exercise. Vet. Clin. Pathol. 2001, 30, 214–218. [CrossRef]
55. de Moffarts, B.; Kirschvink, N.; Art, T.; Pincemail, J.; Lekeux, P. Effect of oral antioxidant supplementation on blood antioxidant status in trained thoroughbred horses. *Vet. J.* 2008, 169, 65–74. [CrossRef]

56. Kienzle, E.; Freismuth, A.; Reusch, A. Double-blind placebo-controlled vitamin E or selenium supplementation of sport horses with unspecified muscle problems. An example of the potential of placebos. *J. Nutr.* 2006, 136, 2045S–2047S. [CrossRef]

57. Williams, C.A.; Carlucci, S.A. Oral vitamin E supplementation on oxidative stress, vitamin and antioxidant status in intensely exercised horses. *Equine Vet. J.* Suppl. 2006, 607–621. [CrossRef]

58. Rey, A.I.; Segura, J.; Arandilla, E.; Lopez-Bote, C.J. Short- and long-term effect of oral administration of micellized natural vitamin E (D-α-tocopherol) on oxidative stress in race horses under intense training. *J. Anim. Sci.* 2013, 91, 1277–1284. [CrossRef]

59. Saastamoinen, M.T.; Juusela, J. Serum Vitamin E Concentration of Horses on Different Vitamin E Supplementation Levels. *Acta Agric. Scand. Sect. A Anim. Sci.* 1993, 43, 52–57. [CrossRef]

60. Meydani, M. Vitamin E. *Lancet* 1995, 345, 170–175. [CrossRef]

61. Evans, W.J. Vitamin E, vitamin C, and exercise. *Am. J. Clin. Nutr.* 2000, 72, 647S–652S. [CrossRef]

62. Blatt, D.H.; Leonard, S.W.; Traber, M.G. Vitamin E kinetics and the function of tocopherol regulatory proteins. *Nutrition* 2001, 17, 799–805. [CrossRef]

63. Sumida, S.; Tanaka, K.; Kitao, H.; Nakadomo, F. Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. *Int. J. Biochem.* 1989, 21, 835–838. [CrossRef] [PubMed]

64. Meydani, M.; Evans, W.J.; Handelman, G.; Biddle, L.; Fielding, R.A.; Meydani, S.N.; Burrill, J.; Fiatarone, M.A.; Blumberg, J.B.; Cannon, J.G. Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. *Am. J. Physiol.* 1993, 264, R992–R998. [CrossRef] [PubMed]

65. Rotkitzki, L.; Logemann, E.; Huber, G.; Keck, E.; Keul, J. α-Tocopherol supplementation in racing cyclists during extreme endurance training. *Int. J. Sport Nutr.* 1994, 4, 253–264. [CrossRef]

66. Sacheck, J.M.; Blumberg, J.B. Role of vitamin E and oxidative stress in exercise. *Nutrition* 2001, 17, 809–814. [CrossRef]

67. Schep, J.M.; Millbury, P.E.; Cannon, J.G.; Roubenoff, R.; Blumberg, J.B. Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. *Free Radiac. Biol. Med.* 2003, 34, 1575–1588. [CrossRef]

68. Duberstein, K.J.; Johnson, S.E.; McDowell, L.R.; Ott, E.A. Effects of vitamin E supplementation and training on oxidative stress parameters measured in exercising horses. *Comp. Exerc. Physiol.* 2009, 6, 17–25. [CrossRef]

69. Finaud, J.; Lac, G.; Filaire, E. Oxidative stress: Relationship with exercise and training. *Sports Med.* 2006, 36, 327–358. [CrossRef]

70. Maughan, R.J.; Burke, L.M.; Dvorak, J.; Larson-Meyer, D.E.; Peeling, P.; Phillips, S.M.; Rawson, E.S.; Walsh, N.P.; Garthie, I.; Geyer, H.; et al. IOC consensus statement: Dietary supplements and the high-performance athlete. *Br. J. Sports Med.* 2018, 52, 439–455. [CrossRef]

71. Littarru, G.P.; Tiano, L. Bioenergetic and antioxidant properties of coenzyme Q10: Recent developments. *Mol. Biotechnol.* 2007, 37, 31–37. [CrossRef]

72. Bentinger, M.; Brismar, K.; Dallner, G. The antioxidant role of coenzyme Q. *Mitochondrion* 2007, 7, S41–S50. [CrossRef]

73. Lass, A.; Sohal, R.S. Electron transport-linked ubiquinone-dependent recycling of alpha-tocopherol inhibits autooxidation of mitochondrial membranes. *Arch. Biochem. Biophys.* 1998, 352, 229–236. [CrossRef]

74. Ostman, B.; Sjödin, A.; Michaelsson, K.; Byberg, L. Coenzyme Q10 supplementation and exercise-induced oxidative stress in humans. *Nutrition* 2012, 28, 403–417. [CrossRef]

75. Sarmiento, A.; Diaz-Castro, J.; Pulido-Moran, M.; Kajarabille, N.; Guisado, R.; Ochoa, J.J. Coenzyme Q10 Supplementation and Exercise in Healthy Humans: A Systematic Review. *Curr. Drug Metab.* 2016, 17, 345–358. [CrossRef]

76. Orlando, P.; Silvestri, S.; Galeazzi, R.; Antonicelli, R.; Marcheggiani, F.; Cirilli, I.; Bacchetti, T.; Tiano, L. Effect of ubiquinol supplementation on biochemical and oxidative stress indexes after intense exercise in young athletes. *Redox Rep.* 2018, 23, 136–145. [CrossRef]

77. Kon, M.; Tanabe, K.; Akimoto, T.; Kimura, F.; Tanimura, Y.; Shimizu, K.; Okamoto, T.; Kono, I. Reducing exercise-induced muscular injury in kendo athletes with supplementation of coenzyme Q10. *Br. J. Nutr.* 2008, 100, 903–909. [CrossRef]

78. Leelarungrayub, D.; Sawattikanon, N.; Klaphajone, J.; Pothongsunan, P.; Bloomer, R.J. Coenzyme Q10 Supplementation Decreases Oxidative Stress and Improves Physical Performance in Young Swimmers: A Pilot Study. *Open Sports Med. J.* 2010, 4. [CrossRef]

79. Diaz-Castro, J.; Guisado, R.; Kajarabille, N.; Garcia, C.; Guisado, I.M.; de Teresa, C.; Ochoa, J.J. Coenzyme Q10 supplementation ameliorates inflammatory signaling and oxidative stress associated with strenuous exercise. *Eur. J. Nutr.* 2011, 51, 791–799. [CrossRef]

80. Sinatra, S.T.; Chopra, R.K.; Jankowitz, S.; Horohov, D.W.; Bhagavan, H.N. Coenzyme Q10 in Equine Serum: Response to Supplementation. *J. Equine Vet. Sci.* 2013, 33, 71–73. [CrossRef]

81. Prosek, M.; Smidovnik, A.; Fir, M.; Strazisar, M.; Andrensek, S.; Wondra, A.G.; Zmitek, J. New Water-Soluble form of Coenzyme Q10 in the Form of an Inclusion Complex with Beta-Cyclodextrin, Process of Preparing, and Use Thereof. U.S. Patent 20,070,202,090,A1, 18 May 2005.

82. Lawrence, L.M. Updates to the nutrient requirements of the horse: NRC 2007 guidelines. In *Current Therapy in Equine Medicine*, 6th ed.; Robinson, N.E., Sprayberry, K.A., Eds.; Elsevier Saunders: St. Louis, MO, USA, 2009; pp. 66–72.

83. Czauderna, M.; Kowalczyk, J.; Marounek, M. The simple and sensitive measurement of malondialdehyde in selected specimens of biological origin and some feed by reversed phase high performance liquid chromatography. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2011, 879, 2251–2258. [CrossRef]
84. Topolovec, M.B.; Kralje, P.; Prosek, M.; Krizman, P.J.; Smidovnik, A.; Svetle, A.N. Endogenous plasma coenzyme Q10 concentration does not correlate with plasma total antioxidant capacity level in healthy untrained horses. Res. Vet. Sci. 2013, 95, 679–677. [CrossRef]

85. Zhao, B.; Tham, S.Y.; Lu, J.; Lai, M.H.; Lee, L.K.; Moochhala, S.M. Simultaneous determination of vitamins C, E and beta-carotene in human plasma by high-performance liquid chromatography with photodiode-array detection. J. Pharm. Pharm. Sci. 2004, 7, 200–204.

86. Sivertsen, T.; Overnes, G.; Osteras, O.; Nymoen, U.; Luder, T. Plasma vitamin E and blood selenium concentrations in Norwegian dairy cows: Regional differences and relations to feeding and health. Acta Vet. Scand. 2005, 46, 177–191. [CrossRef]

87. Pinheiro, J.C.; Bates, D.M. Mixed-Effects Models in S and S-PLUS; Springer: New York, NY, USA, 2000.

88. Brown, H.; Prescott, R. Applied Mixed Models in Medicine, Statistics in Practice; 2nd ed.; John Wiley & Sons: Edinburgh, Scotland, 2006.

89. Jeanes, Y.M.; Hall, W.L.; Ellard, S.; Lee, E.; Lodge, J.K. The absorption of vitamin E is influenced by the amount of fat in a meal and the food matrix. Br. J. Nutr. 2004, 92, 575–579. [CrossRef]

90. Nielsen, F.; Mikkelsen, B.B.; Nielsen, J.B.; Andersen, H.R.; Grandjean, P. Plasma malondialdehyde as biomarker for oxidative stress: Reference interval and effects of life-style factors. Clin. Chem. 1997, 43, 1209–1214. [CrossRef]

91. Sivertsen, T.; Overnes, G.; Osteras, O.; Nymoen, U.; Luder, T. Plasma vitamin E and blood selenium concentrations in Norwegian dairy cows: Regional differences and relations to feeding and health. Acta Vet. Scand. 2005, 46, 177–191. [CrossRef]

92. Finno, C.J.; Valberg, S.J. A comparative review of vitamin E and associated equine disorders. J. Vet. Intern. Med. 2012, 26, 1251–1266. [CrossRef]

93. Pagan, J.D.; Kane, E.; Nash, D. Form and source of tocopherol affects vitamin E status in Thoroughbred horses. Pferdeheikunde 2005, 21, 101–102. [CrossRef]

94. Takanami, Y.; Iwane, H.; Kawai, Y.; Shimomitsu, T. Vitamin E supplementation and endurance exercise: Are there benefits? Sports Med. 2000, 29, 73–83. [CrossRef]

95. Lopez-Lluch, G.; Del Pozo-Cruz, J.; Sanchez-Cuesta, A.; Cortes-Rodriguez, A.B.; Navas, P. Bioavailability of coenzyme Q10 supplements depends on carrier lipids and solubilization. Nutrition 2019, 57, 133–140. [CrossRef] [PubMed]

96. Braun, B.; Clarkson, P.M.; Freedson, P.S.; Kohl, R.L. Effects of coenzyme Q10 supplementation on exercise performance, VO2max, and lipid peroxidation in trained cyclists. Int. J. Sport Nutr. 1991, 1, 353–365. [CrossRef] [PubMed]

97. Cooke, M.; Iosia, M.; Buford, T.; Shelmadine, B.; Hudson, G.; Kerkis, C.; Rasmussen, C.; Greenwood, M.; Leutholtz, B.; Willoughby, D.; et al. Effects of acute and 14-day coenzyme Q10 supplementation on exercise performance in both trained and untrained individuals. J. Int. Soc. Sports Nutr. 2008, 5, 8. [CrossRef] [PubMed]

98. McAnulty, S.R.; McAnulty, L.S.; Nieman, D.C.; Morrow, J.D.; Shooter, L.A.; Holmes, S.; Heward, C.; Henson, D.A. Effect of coenzyme Q10 and alpha-tocopherol administration on their tissue levels in the mouse: Elevation of mitochondrial alpha-tocopherol by coenzyme Q10. Free Radic. Biol. Med. 1999, 26, 1375–1382. [CrossRef] [PubMed]

99. Topolovec, M.B.; Kruljc, P.; Prosek, M.; Krizman, P.J.; Smidovnik, A.; Svetle, A.N. Endogenous plasma coenzyme Q10 concentration does not correlate with plasma total antioxidant capacity level in healthy untrained horses. Res. Vet. Sci. 2013, 95, 679–677. [CrossRef]

100. Thomas, S.R.; Neuzil, J.; Stocker, R. Co-supplementation with coenzyme Q prevents the prooxidant effect of alpha-tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation. Arterioscler. Thromb. Vasc. Biol. 1996, 16, 687–696. [CrossRef]

101. Nielsen, F.; Mikkelsen, B.B.; Nielsen, J.B.; Andersen, H.R.; Grandjean, P. Plasma malondialdehyde as biomarker for oxidative stress: Reference interval and effects of life-style factors. Clin. Chem. 1997, 43, 1209–1214. [CrossRef]

102. Grotto, D.; Maria, L.S.; Valentini, J.; Paniz, C.; Schmitt, G.; Garcia, S.C.; Pomblum, V.I.; Rocha, J.B.T.; Farina, M. Importance of the lipid peroxidation biomarkers and methodological aspects FOR malondialdehyde quantification. Quim. Nova 2009, 32, 169–174. [CrossRef]
110. Spirlandeli, A.L.; Deminice, R.; Jordao, A.A. Plasma malondialdehyde as biomarker of lipid peroxidation: Effects of acute exercise. *Int. J. Sports Med.* 2014, 35, 14–18. [CrossRef]

111. A-Qudah, K.M.; Al-Majali, A.M. Status of biochemical and antioxidant variables in horses before and after long distance race. *Rev. Méd. Vet.* 2006, 157, 307–312.

112. Gul, I.; Gokbel, H.; Belviranli, M.; Okudan, N.; Buyukbas, S.; Basarali, K. Oxidative stress and antioxidant defense in plasma after repeated bouts of supramaximal exercise: The effect of coenzyme Q10. *J. Sports Med. Phys. Fit.* 2011, 51, 305–312.

113. Prior, R.L.; Cao, G. In vivo total antioxidant capacity: Comparison of different analytical methods. *Free Radic. Biol. Med.* 1999, 27, 1173–1181. [CrossRef]

114. Ghiselli, A.; Serafini, M.; Natella, F.; Scaccini, C. Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. *Free Radic. Biol. Med.* 2000, 29, 1106–1114. [CrossRef]

115. Cao, G.; Prior, R.L. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin. Chem.* 1998, 44, 1309–1315. [CrossRef]

116. Serafini, M.; Del Rio, D. Understanding the association between dietary antioxidants, redox status and disease: Is the Total Antioxidant Capacity the right tool? *Redox Rep.* 2004, 9, 145–152. [CrossRef] [PubMed]

117. Apak, R. Current Issues in Antioxidant Measurement. *J. Agric. Food Chem.* 2019, 67, 9187–9202. [CrossRef] [PubMed]

118. Kedzierski, W.; Bergero, D.; Assenza, A. Trends of hematological and biochemical values in the blood of young race horses during standardized field exercise tests. *Acta Vet.* 2009, 59, 457–466. [CrossRef]

119. Kinnunen, S.; Atalay, M.; Hyvypa, S.; Lehmuskero, A.; Hanninen, O.; Oksala, N. Effects of prolonged exercise on oxidative stress and antioxidant defense in endurance horse. *J. Sports Sci. Med.* 2005, 4, 415–421. [PubMed]

120. Lamprecht, E.D.; Williams, C.A. Biomarkers of antioxidant status, inflammation, and cartilage metabolism are affected by acute intense exercise but not superoxide dismutase supplementation in horses. *Oxid. Med. Cell Longev.* 2012, 2012, 920932. [CrossRef]

121. Kruljc, P.; Cebulj-Kadunc, N.; Frangez, R.; Svete, N.N. Changes in blood antioxidant, biochemical and haematological parameters in police horses on duty. *Slov. Vet. Res.* 2014, 51, 119–129.

122. Siqueira, R.F.; Weigel, R.A.; Nunes, G.R.; Mori, C.S.; Fernandes, W.R. Oxidative profiles of endurance horses racing different distances. *Arq. Bras. Med. Vet. Zootec.* 2014, 66, 455–461. [CrossRef]

123. Helgheim, I.; Hetland, O.; Nilsson, S.; Ingjer, F.; Stromme, S.B. The effects of vitamin E on serum enzyme levels following heavy exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* 1979, 40, 283–289. [CrossRef]