Ergothioneine modulates Interleukin-6 Serum concentration in Arabian Stallions following a 2000 m race at maximum speed in a hot-dry environment

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Abstract

This experiment was performed to determine ergothioneine's effect on serum interleukin-6 (IL-6) concentrations after a race of 2000 m in Arabian stallions at the highest possible speed in a hot-dry environment. Twenty-four apparently healthy stallions of the Arabian breed were used. The average weight and age of the horses were 401 ± 32.11 kg and 5.7 ± 0.54 years respectively. The experimental subjects were split into three units of eight stallions each. Group 1 (EXEN) was neither treated with ergothioneine nor exercised. Group II (EXEC) was not treated with ergothioneine but was exercised. The third group (EXEE) was treated with ergothioneine *per os* at a dose rate of 0.02 mg/kg daily for one month prior to the experiment. Meteorological parameters of the study site were recorded using a dry and wet-bulb thermometer. The stallions in the EXEC and EXEE groups were made to run a race of 2000 m at the highest possible speed by trained riders. Samples of blood of 10 ml were collected from all stallions before the experiment and immediately after the experiment and two hours post-experiment. The hematological parameters and concentration of IL-6 were determined in all samples. The concentration of IL-6 was found to be higher significantly in the EXEE group suggesting a modulatory role of ergothioneine. Therefore, it was concluded that ergothioneine enhanced IL-6 following exercise and would be beneficial to stallions during exercise.

Keywords: Ergothioneine; Interleukin 6; Hematological parameters; Exercise; Arabian stallions

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Abbreviations
IL-6: Interleukin 6
EXEN: Not treated, not exercised
EXEC: Not treated but exercised
EXEE: Treated and exercised
ROS: reactive oxygen species

RH: relative humidity
THI: temperature humidity index
DBT: dry bulb temperature

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Introduction

The body's homeostatic response is tremendously affected by rigorous exercise and is negatively influenced by high ambient temperatures and relative humidity [1].

Although several sources of IL-6 have been identified such as T cells, B cells, macrophages, neutrophils, monocytes, keratinocytes, fibroblasts, endothelial cells, epithelial cells, osteoblasts, chondrocytes, adipocytes, and mesangial cells, the majority of the IL-6 that is produced during exercise and seen in the peripheral blood comes from contracting muscles [2]. Exercise intensity and duration determine how much of the IL-6 response is produced [3]. Muscle contractions and IL-6 synthesis may be connected by several mechanisms and theories: Transcriptional factors that control the production of IL-6 can be activated by alterations in the homeostasis of calcium, decreased availability of glucose, and enhanced free radicals generation [4-6]. Leukocytes, the hepatic tissue, fatty tissue, and the interaction of the hypothalamus, pituitary, and adrenal glands, are all modulated by IL-6 and may all affect how the body responds to exercise in terms of metabolism and immunity. However, for a marked systemic IL-6 response to occur, a significant amount of muscle tissue must be exercised [7]. Intrleukin-6 also known as a myokine possesses a significant anti-inflammatory ability in its effects as a myokine. It significantly increases during exercise and is present in the blood before other cytokines. It is believed that it acts as a hormone to increase extracellular substrates during exercise [8].

Animals cannot synthesize ergothioneine (ERG), an extremely stable, naturally occurring nutraceutical that can only be obtained through their food. It is produced by *Actinomycetales*

bacteria and non-yeast fungi. The greatest amounts of L-ergothioneine are found in mushrooms, especially in species like *Boletus edulis* and *Pleurotus ostreatus*. L-ergothioneine being a potent antioxidant may enhance the synthesis of IL-6 thereby quickly promoting the repair of damaged tissues. It is eliminated from the bloodstream and accumulates in cells and tissues following damage and oxidative stress [9]. It is swiftly absorbed by tissues by an exceptional transporter known as organic cation transporter new type 1 (OCTN-1), which plays distinct and significant functions in cellular processes [10-11]. The rate at which cells collect it suggests that ERG serves a critical physiological role [12]. Ergothioneine has two major functions: it regulates energy and shields tissue from oxidative and inflammatory harm. Ergothioneine does not oxidize at the usual body pH, making it a highly stable antioxidant. Moreover, it prevents the generation of hydroxyl radicals from ferrous (Fe^{2+}) ions and hydrogen peroxide [13]. Ergothioneine combats reactive oxygen species produced during physical activity, such as O^{2-}, H_2O_2, $\cdot OH^-$, and O_2, functioning both as a selfless antioxidant and as a modulator of the cellular antioxidant defense and also modulating cytokine such as IL-6, thereby influencing cellular responses and functions [11].

The diseases rheumatoid arthritis and Crohn's disease are two long-standing inflammatory disorders that have been linked to changes in the gene coding for ergothioneine transporter (OCTN1) [14-15]. Higher erythrocyte and monocyte concentrations of EGT were discovered in individuals with less inflammation, and these levels were strongly linked with the expression of ergothioneine transporter mRNA in specific body cells [16].

This experiment aims to ascertain the effect of ergothioneine on the myokine, interleukin-6, after an exercise of 2000 m at the highest possible speed in Arabian stallions.

Results

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The meteorological indices of the site of the study in the hot-dry season are shown in Table 1 The dry-bulb temperature (DBT) rose ($P < 0.05$) from 22.6 ±1.23 ^{o}C at 6 a.m. in the morning to 38.6 ± 6.53 ^{o}C at mid-day. The humidity index rose ($P < 0.05$) from 64.4 ± 2.34 % at 6 a.m. to 74.3 ± 6.73 % at mid-day (12.00 h). The temperature-humidity index increased from 76.41± 0.56 at 06.00 h in the morning to 83.36 ± 4.53 at mid-day (12.00 h).

Table 1 Meteorological Parameters of the Experimental Site during the Hot-Dry Season

Time of Day	Dry-Bulb Temperature (^{o}C)	Relative Humidity (%)	Temperature-Humidity Index
06.00	22.6 ± 1.23[a]	64.4 ± 2.34[a]	76.41 ± 0.56[a]
	(22 - 24)	(63 – 68)	(68.71 - 83.65)
12.00	38.6 ± 6.53[b]	74.3± 6.73[b]	83.36 ± 4.53[b]
	(37-39)	(72 - 78)	(81.32 - 89.01)
18.00	36.5 ± 0.17	78.8 ± 5.98	83.24 ± 3.49
	(36-37)	(76 - 81)	(83.21 - 84.95)
Overall Mean ± SEM	37.22 ± 4.17	75.19 ± 5.98	81.45 ± 5.18
	(36.11 - 37.33)	(73.65 – 81.11)	(78.11-84.76)

[a,b]Means for the same column having different superscript letters are significantly ($P < 0.05$) different .Values in Parentheses are Minimum - Maximum.

The findings of hematological indices are shown in Table 2. A higher leucocyte count (8.73 ± 0.94 × 10^{9}) was obtained in EXEC group compared to the count of 4.03 ± 0.14 × 10^{9} recorded in the EXEE group. The neutrophil count of 4.04 ± 3.09 × 10^{9} recorded in the EXEE group was higher ($P < 0.05$) than the value (2.14 ± 0.63 × 10^{9}) obtained in the EXEE stallions after the race.

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The value of stress index recorded in EXEC (4.17 ± 0.69) was higher than the value of 2.97 ± 0.13 recorded in the EXEE horses.

Table 2 Haematological Parameters of Stallions in the Hot-Dry Season

Parameters	Time	NEXE	EXEC	EXEE
Leucocytes ($\times 10^9$/L)	Pre-exercise	3.21 ± 0.13	4.41 ± 0.18	4.82 ± 0.86
	Post-exercise	3.33 ± 0.24	8.73 ± 0.94[a]	4.03 ± 0.14[b]
Neutrophil ($\times 10^9$/L)	Pre-exercise	2.53 ± 0.11	2.83 ± 0.46	2.62 ± 0.76
	Post-exercise	2.42 ± 0.13	4.04 ± 3.09[a]	2.14 ± 0.63[b]
Lymphocytes ($\times 10^9$/L)	Pre-exercise	1.32 ± 0.21	1.72 ± 0.21	1.80 ± 0.19
	Post-exercise	1.48 ± 0.42	1.69 ± 0.42	1.63 ± 0.57
Monocytes ($\times 10^9$/L)	Pre-exercise	0.11 ± 0.05	0.17 ± 0.02	0.17 ± 0.03
	Post-exercise	0.17 ± 0.03	0.18 ± 0.04	0.21 ± 0.02
Erythrocytes ($\times 10^{12}$/L)	Pre-exercise	6.12 ± 0.13	7.42 ± 0.23	6.82 ± 0.37
	Post-exercise	15.22 ± 3.74	11.63 ± 1.54	12.22 ± 0.68
Total Protein (g/dl)	Pre-exercise	4.4 ± 0.13	3.9 ± 0.16	4.35 ± 0.21
	Post-exercise	5.1 ± 0.17	6.77 ± 0.56	7.11 ± 0.58
Packed Cell Volume (%)	Pre-exercise	32.13 ± 7.25	29.07 ± 0.26	29.15 ± 1.21
	Post-exercise	35.42 ± 0.14	45.42 ± 0.24[a]	54.42 ± 3.87[b]
Haemoglobin (g/dl)	Pre-exercise	9.71 ± 0.36	8.71 ± 0.36	7.65 ± 0.31
	Post-exercise	9.95 ± 3.11	10.27 ± 2.77	8.93 ± 2.83
Neutrophil/Lymphocyte ratio	Pre-exercise	1.91 ± 0.88	1.65 ± 0.32	1.46 ± 0.64
	Post-exercise	2.02 ± 0.03	4.17 ± 0.69[a]	2.97 ± 0.23[b]

[a,b]Means for the same row having different superscript letters are significantly ($P < 0.05$) different.

Key: NEXE = Not administered with ergothioneine, not exercised
EXEC = Not administered with ergothioneine but exercise
EXEE = Administered with ergothioneine before exercise

The IL-6 concentrations recorded in this study are shown in Figure 1. Concentrations of IL-6 obtained post-exercise were significantly higher in the EXEE stallions than the EXEC stallions.

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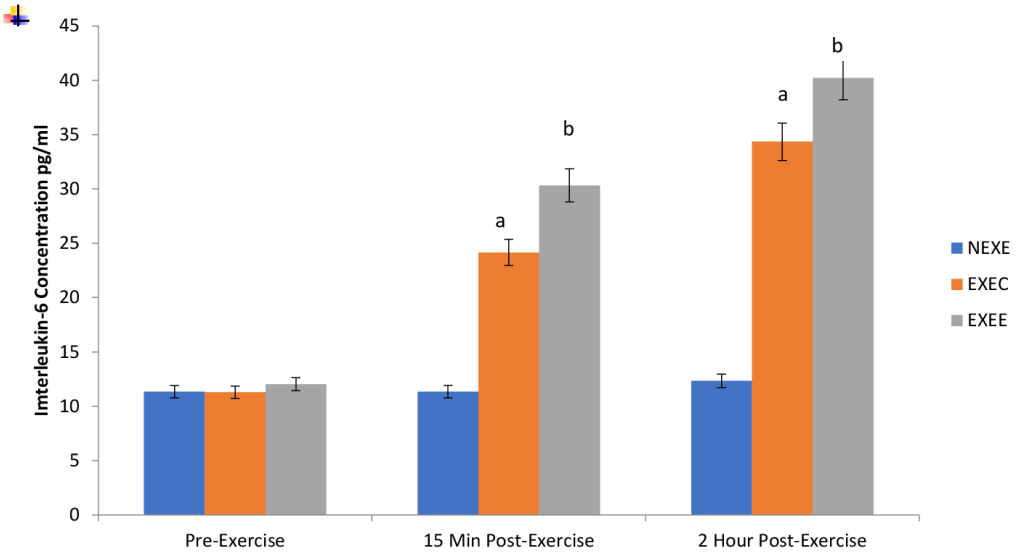

Figure 1.Concentration of Interleukin-6 in the Stallions

Key: NEXE = Not administered with ergothioneine, not exercised
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Discussion

The environmental temperature, humidity index, and temperature-humidity index of the experimental site in the hot-dry season were all found to be relatively high. The study's overall mean DBT of 37.22 ± 4.17 ºC was higher than the 20–25 ºC range considered to be the thermoneutral range zone for horses [17-19]. Horses experience heat stress when the ambient temperature is higher than the thermoneutral zone because of increased metabolism, enhanced heat losses via skin evaporation, and lowered thermal insulation. The RH value (75.19 ± 5.98%) recorded was also higher than the 70% level recommended for horses [20]. Elevated DBT and RH values in this current experiment suggest that the horses experienced thermal stress. High DBT and RH make it challenging for sweat to evaporate from the skin's surface because the air is already moist. The horse is therefore put under heat stress by this occurrence. According to dos Santos et al. [21], the THI, a measure of heat load, was extremely high in this study, indicating that the stallions were under stress due to the hot temperatures in the study area. Elevated THI levels found in this study indicated the need for interventions, including the installation of fans in the stallions' stables and the administration of supplements to the horses, to lessen the deleterious effects of heat stress. Exercise causes a horse to gradually lose heat from its muscles and internal organs into the blood, which worsens the effects of heat stress on the stallions' locomotor system and lowers performance [22].

According to Faria et al. [23], the stress index (neutrophil-lymphocyte ratio), which is an indicator of inflammation and stress, was higher in the EXEC stallions than in the EXEE stallions, suggesting that the treatment with ergothioneine before exercise reduced the stress of exercise and

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lowered inflammatory response. Better stress management and a lowered inflammatory response are indicated by a lower stress index (neutrophil-lymphocyte ratio).

It is unclear how the skeletal muscle responds to exercise-induced inflammation and injury by coordinating an adaptation mechanism that protects the muscle from future harm, known as the repeated bout effect (RBE) [24] although IL-6, which has both pro- and anti-inflammatory action, plays a crucial role. IL-6 is a myokine and is produced and expressed by the muscles than other cytokines such as TNFα and IL1β. It sends out an alert signal when there is tissue damage due to exercise [8]. The anti-inflammatory impact typically occurs after a brief but intense exercise, as demonstrated in this study [25]. IL6's anti-inflammatory effects are brought on by the classical signaling mechanism [8]. More than any other cytokine, the blood concentration of IL-6 rises after intense exercise [25]. It has been proven that IL-6 has a role in regulating the early inflammatory response brought on by exercise in horses [26]. After exercise, interleukin-6 (IL-6) is released from the contracting skeletal muscles into the blood stream and metabolism is characterised by rapid increases in plasma levels, with a peak few hours after exercise, and a gradual return to basal levels within 48 hours. IL-6 plays a role in regulating energy intake during exercise, acting as a "smart meter" to signal the release of energy substrates from liver and fatty tissue [25]. Although having a low affinity for IL-6, soluble glycoprotein 130 (sgp130) possesses a marked affinity for the IL-6/sIL-6 complex, inhibiting the inflammatory action of IL6 after brief, intense exercise as observed in this study (21). According to a study, myocytes and macrophages have quite different signaling pathways for interleukin-6 in the exercising skeletal muscle after a period of intensive exercise [27]. It appears that muscular interleukin-6 expression is controlled by a connection of signaling events, including the Ca^{2+}/NFAT and glycogen/p38 MAPK pathways, in contrast to macrophages IL-6 signaling, which depends on eliciting the nuclear factor kappa (BNF-B) signaling pathway

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[28]. Hence, IL-6 signaling in macrophages or monocytes results in inflammation, whereas the activation of IL-6 and its signaling in skeletal muscle after exercise is completely different from an earlier TNF response or NF-B activation and reduces inflammation [29]. Muscles are known to produce and release IL-6, which has been linked to the regulation of metabolism and the acceleration of satellite cell regeneration and proliferative activities [30]. Interleukin-6 is a short-term energy allocator that is also released from muscle as a result of reduced energy content. Interleukin-6 improves muscular energy uptake during exercise and releases cellular energy content through lipolysis [31]. We concluded that the greater levels of IL-6 after intense exercise may inhibit the excessive production of type 1 pro-inflammatory cytokines, hence lowering inflammation. An important enzyme involved in inflammation called myeloperoxidase (MPO) has been demonstrated to be inhibited by ergothioneine. Moreover, it is widely known for terminating MPO-based reactive substances more quickly than glutathione and vitamin C. This includes HOCl [9]

Many investigations on this special molecule have revealed that it may lessen underlying disorders and tissue damage in various tissues, including the skeletal muscles [9]. Divalent metal ions like Fe^{2+} and Cu^{2+} can be bound to ergothioneine with high stability constants [12]. By creating redox-inactive compounds with ergothioneine, this metal ion chelation stops the deterioration of biomolecules. Ergothioneine could prevent future oxidative stress, which would reduce harm to muscles, other tissues, and organs (especially the brain) [32]. While proinflammatory cytokines' expression is influenced by ergothioneine, it is possible that this compound could help break the cycle of hyperinflammation that is caused by macrophage activation and hyperferritinemia [33-34]. The findings of this study agree with the study of Steensberg et al [35] who reported that exercise can cause an increase in interleukin-6, which can be explained by the synthesis of

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interleukin-6 in contracting human skeletal muscles. Ergothioneine effectively targeted reactive oxygen species produced during the exercise, such as O^{2-}, H_2O_2, ·hydroxyl ion and oxygen, functioning both as a self-sacrificing antioxidant and as a modulator of the cellular antioxidant defense and immune systems, thereby influencing the cellular redox state. Once the cellular redox state was established, it enhanced the optimal activity of S-Adenosyl-methionine (SAM) synthase. SAM plays a crucial role in maintaining normal cellular function and survival, being involved in three essential metabolic pathways: transmethylation, trans-sulfuration, and polyamine synthesis, all of which are vital for normal cellular function and stability.

Conclusion

It can therefore be concluded that ergothioneine administration to stallions before exercise will enhance some hematological parameters and the synthesis of IL-6.

Materials and Methods

Experimental Animals

We used 24 untrained, clinically healthy Arabian stallions aged 5.7 ± 0.54 (5-6 years) years, with an average weight of 401 ± 32.11 kilograms (395 to 404 kg). The stallions were obtained from a royal stable and were only used for pleasure riding. The stallions were divided into three groups of eight each. Group I (NEXE), did not engage in exercise and was not administered ergothioneine, the second group (EXEC), engaged in exercise but was not administered ergothioneine, and the third group (EXEE), received ergothioneine before exercise.

The housing for the stallions was constructed using cement bricks, a concrete floor, corrugated iron roofing sheets, and a wooden ceiling. The horses underwent two weeks of preconditioning.

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The horses were fed with hay and their meal was augmented with groundnut bran. They had unlimited access to water. The stallions were checked for intestinal helminths, and all the infected ones were treated using albendazole (5 mg/kg body weight; Jubaili Agrotec, Kano, Nigeria).

Determination of Thermal Environmental Parameters

The relative humidity (RH) and dry-bulb temperature (DBT) values were ascertained by deploying a dry and wet-bulb thermometer manufactured by Mark, England. The formula postulated by Hartmann et al. [17], stated below was used to calculate the Temperature Humidity Index (THI).

$$THI = (DBT \times 0.8) + \{(RH/100 \times (DBT-14.4) + 46.4\}$$

Blood Sampling

Before the experiment, the site of the collection was cleaned with a clean piece of cotton dipped in an alcohol solution. Samples of blood (10 mL) were obtained from each stallion 15 minutes after the exercise and 2 hours after the exercise using 18-gauge needles. Jugular venipuncture was used to get the blood samples, and they were subsequently dispensed into both plain vacuum containers and vacuum containers with the anticoagulant potassium ethylenediaminetetraacetic acid (EDTA). The hematological parameters and IL-6 levels of the samples of blood were determined in the physiological research laboratory after the samples were conveyed to the laboratory using a box packed with ice. The stallions were fasted for about three hours and were fed only after the experiment.

Administration of Ergothioneine to Stallions

Ergothioneine manufactured by Oxis International, Los Angeles, California, USA was given orally to each horse in the EXEE group four weeks before the experiment at a dose of 0.2 mg/kg. In the

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morning, ergothioneine was given before eating. Ergothioneine was placed in warm water and aspirated using a correct-sized syringe. The solution was given orally to the restrained horse by putting the syringe into the mouth's corner and depressing the plunger to dispense the medication into the animal's mouth. The horse's head was then slightly raised to ensure that the entire solution had been swallowed.

Exercise Protocol

Following the treatment of the stallions in the EXEE group, each of them was mounted by an appropriately kitted and trained rider with an average weight of 70.56 ± 4.23 kg and exercised at 2000 m at the highest possible speed using a standard race track.

Determination of Haematological Parameters

Using a standardized automatic analyzer for veterinary use (KT-6610 VET) manufactured in Jiangsu, China) the following parameters were determined count of erythrocytes, count of leucocytes, haematocrit, and concentration of haemoglobin.

Determination of Serum Interleukin-6 Concentration

The Cloud-Clone Corporation (Houston, USA) horse IL-6 ELISA kit a highly sensitive kit that shows detectable values in serum was deployed to evaluate the serum content of IL-6. To obtain the serum, the whole blood was drawn, permitted to clot, and subsequently centrifuged to isolate the serum from blood cells and other elements. The clear, yellowish liquid that remains is the serum. An anti-interleukin-6 antibody which has been coated on the microplate was enclosed with this kit. The antibody conjugated with biotin unique for IL-6 was then placed in the appropriate microplate wells with the standards. Then each microplate well was added with horseradish peroxidase conjugated with avidin and placed in an incubator. Only wells that had interleukin-6,

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antibody conjugated with biotin, and Avidin conjugated enzyme altered its colour after the inclusion of Tetramethylbenzidine (TMB) solution subtrate. A solution of sulphuric acid was used to terminate the substrate-enzyme reaction, and the alteration in colour was evaluated spectrophotometrically at 450 nm wavelength. The optical density of the samples was then compared to the standard plot to ascertain the IL-6 content of the samples.

Data Analyses

The data obtained from this experiment was expressed as mean ± standard error of the mean. The statistical test Shapiro-Wilk was used to determine whether the data was normally distributed. The data was found to be normally distributed. The statistical test of one-way analysis of variance (ANOVA) was deployed to analyze the data after which it was subjected to Tukey's post hoc test. Only values of $p \leq 0.05$ were considered significant. The Graph Pad Prism (version 5.3) manufactured by (GraphPad Software, Inc.) was used.

Authors' Contributions: ASA and JOA designed the experiment. ASA, JOA, PIR, and TA developed and edited the manuscript. DAA and ASA performed the research and analysis of the data

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Conflicts of Interest

The authors hereby declare that they have no competing interests

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References

1. Travers G, Kippelen P, Trangmar SJ, González-Alonso J. Physiological Function during Exercise and Environmental Stress in Humans-An Integrative View of Body Systems and Homeostasis. Cells, 2022;11(3):383. https://doi.org/10.3390/cells110303831.
2. Ritten S, Schusser GF, Abraham G, Schrödl W. Release kinetics of tumor necrosis factor-α and interleukin-1 receptor antagonist in the equine whole blood. BMC Vet Res, 2016;12(1):117-122. https://doi.org/10.1186/s12917-016-0742-4
3. Suzuki K. Cytokine Response to Exercise and Its Modulation. Antioxidants. 2018; 7(1):17. https://doi.org/10.3390/antiox7010017
4. Gjevestad GO, Hamarsland H, Raastad T, Ottestad I, Christensen JJ, Eckardt K, Drevon CA, Biong AS, Ulven SM, Holven KB. Gene expression is differentially regulated in skeletal muscle and circulating immune cells in response to an acute bout of high-load strength exercise. Genes Nutr, 2017;3:12-18. doi: 10.1186/s12263-017-0556-.
5. Vijayaraghava A, Doreswamy V. Exercise and the cytokines –interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α): A review. Annals Med Physiol, 2017;1(1): 3-8. DOI:10.23921/AMP.2017V1I1.263485
6. Suzuki K, Hayashida H. Effect of Exercise Intensity on Cell-Mediated Immunity. Sports 2021; 9(1), 8. https://doi.org/10.3390/sports9010008
7. Morettini M, Palumbo MC, Sacchetti M, Castiglione F, Mazza C. A system model of the effects of exercise on plasma Interleukin-6 dynamics in healthy individuals: Role of skeletal muscle and adipose tissue. PLoS ONE, 2017;12(7): e0181224, doi: 10.1371/journal.pone.0181224
8. Christian M, Nussbaum MA. An Exploratory Study of the Effects of Occupational Exposure to Physical Demands on Biomarkers of Cartilage and Muscle Damage. J Occup and Env Hyg 2015;12(2):138-144. DOI:10.1080/15459624.2014.955181
9. Cheah IK, Halliwell B. Could Ergothioneine Aid in the Treatment of Coronavirus Patients? Antioxidants. 2020; 9(7):595. https://doi.org/10.3390/antiox9070595
10. Bazela K, Solyga-Zurek A, Debowska R, Rogiewicz K, Bartnik E, Eris I. L- ergothioneine protects skin cells against ultraviolet ray-induced damage - A Preliminary study. Cosmetics 2014; 1:51-60. DOI: 10.3390/cosmetics1010051
11. Cheah IK, Tang Y, Ye P, Yew TSZ, Lim KHS, Halliwell B. Liver ergothioneine accumulation in a guinea pig model of non-alcoholic fatty liver disease. A possible mechanism of defence? Free Rad Res 2016; 50(1):14-25. DOI: 10.3109/10715762.2015.1099642.
12. Paul BD, Snyder SH. The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. Cell Death and Differentiation, 2009;17: 1-7. DOI: 10.1038/cdd.2009.163

Uncorrected Proof

13. Carpentieri A, Barboza GD, Areco V, Lopez MP, Talamoni NT. New perspectives in melatonin uses. Pharmacol Res, 2012; 65: 437–444. DOI: 10.1016/j.phrs.2012.01.003
14. Asahi T, Wu X, Shimoda H, Hisaka S, Harada E, Kanno T, Nakamura Y, Kato, Osawa T. A mushroom-derived amino acid, ergothioneine, is a potential inhibitor of inflammation-related DNA halogenation. J Biosci Biotech Biochem 2016; 80(2):313-317. doi: 10.1080/09168451.2015.1083396.
15. Peltekova VD, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van B, Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat Genet,2004; 36(5): 471-5
16. Pochini L, Michele G, Mariafrancesca S, Lara C, Gilda P, Cesare I. "OCTN1: A Widely Studied but Still Enigmatic Organic Cation Transporter Linked to Human Pathology and Drug Interactions" Inter J Molecul Sci 2022; 23(2): 914. https://doi.org/10.3390/ijms23020914
17. Hartmann E, Hopkins R.J, vonBrömssen C, Dahlborn K. 24-h sheltering behaviour of individually kept horses during Swedish summer weather. Acta Vet Scand, 2015;57: 45-54. DOI: 10.1186/s13028-015-0135-x
18. Morgan K, Ehrlemark A, Sallvik K. Dissipation of heat from standing horses exposed to ambient temperatures between - 3°C and 37°C. J Therm Biol, 1997;22: 177-186. https://doi.org/10.1016/S0306-4565(97)00007-7
19. Wallsten H, Olsson K, Dahlborn K. Temperature regulation in horses during exercise and recovery in a cool environment. Acta Vet Scand, 2012; 54(1): 42-48. DOI: 10.1186/1751-0147-54-42
20. Commission International Du Genie Rural. (1984). Report of Working Group of of Animal Houses, Scottish Farm Buildings Investigation Unit, Craibstone, Bucksburn, Aberdeen, , Scotland for Commission Internationale du Genie Rural (International Commission of Agricultural Engineering).
21. dos Santos GB, de Oliveira AG, Marcondes MC, Areas MA. Long-term Leucine Supplementation Improves Metabolic but not Molecular Responses in the Skeletal Muscle of Trained Rats Submitted to Exhaustive Exercise. J Ame Coll Nutri, 2017;36: 81-87.
22. Hanzawa K, Hiraga A, YoshidaY, Hara H, Kai M, Kubo K, Watanabe S. Effect of exercise on plasma haptoglobin composition in control and splenectomized Thoroughbred horses. J Equi Sci, 2002;13(3): 89-92.
23. Faria FC, Fernandes PC, Silva MJB, Vladmir CL, Fontes W, Freitas-Junior R, Eterovic AK Forget P. The neutrophil-to-lymphocyte ratio: a narrative review. Ecancer, 2016;10: 702-714. DOI: 10.3332/ecancer.2016.702
24. Giuriato G, Venturelli M, Matias A, Soares EMKV, Gaetgens J, Frederick KA, Ives SJ Capsaicin and Its Effect on Exercise Performance, Fatigue and Inflammation after Exercise. Nutrients, 2022;14:232. https:// doi.org/10.3390/nu14020232
25. Witkowska-Piłaszewicz O, Bąska P, Czopowicz M, Żmigrodzka M, Szarska E, Szczepaniak J, Nowak Z, Winnicka A, Cywińska A. Anti-Inflammatory State in Arabian Horses Introduced to the Endurance Training. Animals,2019;9(9):616. DOI: 10.3390/ani9090616
26. Cywinska A, Turło A,Witkowski L, Szarska E, Winnicka A. Changes in blood cytokine concentrations in horses after long-distance endurance rides. Med Weter, 2014;70: 568–571.

Uncorrected Proof

27. Stefan R J. IL-6 Trans-Signaling via the Soluble IL-6 Receptor: Importance for the Pro-Inflammatory Activities of IL-6. International J Biol Sci, 2012; 8(9):1237. https://doi.org/10.7150/ijbs.4989
28. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harbor. Perspect Biol, 2014;6(10):016295. DOI: 10.1101/cshperspect. a016295
29. Brandt C, Pedersen BK. "The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases". J Biomed Biotech. 2010;520258. doi:10.1155/2010/520258.
30. Pedersen BK. Muscular interleukin-6 and its role as an energy sensor. Med Sci Sports Exerc. 2012; 4:392–396.
31. Kistner TM, Pedersen BK, Lieberman DE. Interleukin 6 as an energy allocator in muscle tissue. Nat Metabol, 2022; 4(2), 170–179. https://doi.org/10.1038/s42255-022-00538-4
32. Kerleya RN, McCarthya C, Kellb DB, Kenny LC. The potential therapeutic effects of ergothioneine in pre-eclampsia. Free Rad Biol Med 2018;117: 145–157. DOI: 10.1016/j.freeradbiomed.2017.12.030
33. LaVigne EK Jones AK, Sanchez-Londono A, Schauer AS, Patterson DF, Nadeau JA, Reed SA. Muscle growth in young horses: Effects of age, cytokines, and growth factors. J Ani Sci, 2015; 93(12): 5672–5680. https://doi.org/10.2527/jas.2015-9634
34. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, Febbraio M, Saltin B. Searching for the exercise factor—is IL-6 a candidate. J Muscle Res Cell M, 2003;24:113–119. doi: 10.1023/a:1026070911202.
35. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. J Physiol. 2000;15;529 Pt 1(Pt 1):237-242. doi: 10.1111/j.1469-7793.2000.00237. x.
36. Li YS, Fujihara H, Fujisawa K, Kawai K. Effects of ergothioneine on oxidative DNA damage and immune response induced by circadian rhythm disturbance in mice. J Clin Biochem Nutr. 2025;76(2):117-124. doi: 10.3164/jcbn.24-220.

Corrected proof