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IMPACT OF PHYSICAL EXERCISE ON ERYTHROCYTE MEMBRANE STABILITY IN MALE AND FEMALE SPORT HORSES

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Erythrocytes play a vital role in transporting oxygen and ensuring adequate tissue perfusion. Their structural and functional integrity is essential for optimal athletic performance in horses. Physical exercise can subject red blood cells to mechanical, osmotic and oxidative stress, which can potentially reduce membrane stability and promote haemolysis.

This study investigated the effects of moderate exercise on the resistance of erythrocytes to acid-induced haemolysis in 17 clinically healthy Holsteiner sport horses, paying particular attention to sex-specific differences. Blood samples were collected before and immediately after a standardised one-hour exercise protocol, and erythrocyte susceptibility to 0.2N HCl was assessed spectrophotometrically. Haemolysis kinetics revealed a biphasic pattern: a rapid initial lysis phase (0.5-10 minutes) was followed by slower progression to complete haemolysis (25-30 minutes).

Exercise significantly increased erythrocyte fragility in both sexes, with stallions showing slightly higher resistance at rest. These findings suggest that physical exertion temporarily compromises the integrity of the erythrocyte membrane, primarily affecting the most vulnerable subpopulation.

The study highlights the importance of considering physiological status and sex when evaluating erythrocyte stability, and suggests that acid-induced haemolysis could be used to monitor equine athletic performance and recovery.

Keywords: erythrocytes, acid-induced haemolysis, exercise physiology, oxidative stress, sport horses, sex differences, membrane stability

ВПЛИВ ФІЗИЧНИХ НАВАНТАЖЕНЬ НА СТІЙКІСТЬ ЕРИТРОЦИТАРНИХ МЕМБРАН У КОБИЛ ТА ЖЕРЕБЦІВ СПОРТИВНИХ КОНЕЙ

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Еритроцити відіграють ключову роль у транспорті кисню та забезпеченні адекватного кровопостачання тканин. Їхня структурна та функціональна цілісність є необхідною для оптимальної спортивної продуктивності коней. Фізичне навантаження може піддавати еритроцити механічному, осмотичному та окиснювальному стресу, що потенційно знижує стабільність мембрани та сприяє гемолізу.

У цьому дослідженні ми оцінювали вплив помірного фізичного навантаження на стійкість еритроцитів до кислотоіндукованого гемолізу у 17 клінічно здорових спортивних коней голштинської породи, приділяючи особливу увагу відмінностям між статями. Зразки крові збирали до та відразу після стандартизованого годинного тренувального протоколу, а чутливість еритроцитів до 0,2N HCl оцінювали спектрофотометрично. Кінетика гемолізу продемонструвала біфазний характер: швидка початкова фаза лізису (0,5-10 хвилин) чергувалася зі сповільненою прогресією до повного гемолізу (25-30 хвилин). Фізичне навантаження значно підвищувало крихкість еритроцитів у обох статей, при цьому у жеребців спостерігалася трохи вища стійкість у спокої. Ці результати свідчать про те, що фізичне навантаження тимчасово порушує цілісність мембрани еритроцитів, головним чином впливаючи на найбільш вразливу субпопуляцію.

Дослідження підкреслює важливість врахування фізіологічного стану та статі при оцінці стійкості еритроцитів та вказує на можливість використання кислотоіндукованого гемолізу для моніторингу спортивної працездатності та відновлення коней.

Ключові слова: еритроцити, кислотоіндукований гемоліз, фізіологія тренінгу, окиснювальний стрес, спортивні коні, відмінності за статтю, стабільність мембран

Introduction. Erythrocytes play a central role in oxygen transport and tissue perfusion, and maintaining their structural and functional integrity is critical for optimal athletic performance in horses (Thorn B. et al., 2020). Physical exercise subjects erythrocytes to various physiological stresses, such as mechanical shear, osmotic fluctuations, oxidative stress, and changes in pH and temperature. These stresses can temporarily compromise membrane stability and promote haemolysis (Sentürk U. K. et al., 2001; Obeagu E. I. et al., 2024). Previous studies have shown that susceptibility to osmotic and chemical stress varies depending on exercise intensity and duration, as well as sex, breed, and training status (Cywińska A. et al., 2011; Andriichuk A. et al., 2016). Recent findings also suggest that iron metabolism and ferritin levels may influence

erythrocyte turnover and oxidative resilience in sport horses, adding another layer of complexity to red blood cell dynamics (Kiełbik P. and Witkowska-Piłaszewicz O., 2025).

Acid-induced haemolysis using standardised HCl exposure is a classical and reliable method of evaluating the stability of erythrocyte membranes and of assessing the effects of oxidative and metabolic stress on red blood cells. This method provides valuable insights into how erythrocytes respond dynamically to exercise-induced stressors and enables comparisons to be made across different experimental groups (Ivanov I. T., 1999, 2001; Husakouskaya E. V. et al., 2024). Furthermore, dietary interventions such as polyunsaturated fatty acid (PUFA) supplementation have been demonstrated to influence erythrocyte fragility and increase resistance to osmotic and oxidative stress. This could potentially enhance the overall physiological performance of athletic horses (Bazzano et al., 2015). PUFA supplementation has also been linked to reduced post-exercise lactate accumulation and improved antioxidant status, suggesting multifaceted benefits for equine athletes under high-intensity workloads (Savignone C. et al., 2019).

Despite extensive research on exercise-induced haemolysis, the interplay between sex, physiological status and oxidative stress on erythrocyte acid resistance remains incompletely understood. Understanding these interactions is essential for optimising training regimens, improving recovery strategies and safeguarding the health and performance of equine athletes. Recent studies on ponies and endurance horses suggest that oxidative stress biomarkers, such as 2-thiobarbituric acid reactive substances (TBARS) and oxidatively modified proteins, could be sensitive indicators of erythrocyte vulnerability during exercise (Tkachenko H. et al., 2020). This study therefore aimed to investigate the effects of moderate exercise on erythrocyte resistance to acid-induced haemolysis in sporting stallions and mares, paying particular attention to the influence of sex, exercise-induced oxidative stress and potential adaptive mechanisms associated with training.

Materials and methods.

Horses. The study was conducted in accordance with current European Union legislation and ethical standards, as set out in Council Directive 2010/63/EU on the protection of animals used for scientific purposes. Ethical approval was obtained from the Local Ethics Committee for Animal Experimentation in Gdansk University, Poland and the T. H. Shevchenko National University “Chernihiv Colehium”, Chernihiv, Ukraine.

The study included 17 clinically healthy Holsteiner sport horses, aged 6-12 years, with no signs of pathology. The horses were housed at the 'Wechta' Equestrian Club in Rosnowko, Poland, under standardised management conditions. The horses actively participated in international-level show jumping competitions, including CCI** and CCI*** events. All of the horses came from prominent sport horse lineages and pedigrees, including notable sires such as Contender, Caletto I, Capitol I and Quidam de Revel, which reflects their elite breeding and performance potential. All horses were maintained on identical feeding regimens throughout the study. Water was available ad libitum and the housing conditions, including stall dimensions, bedding type and turnout frequency, were consistent for all animals. Prior to the study, all horses had undergone long-term structured training, including flatwork and jumping exercises, for several years.

All horses underwent a comprehensive clinical evaluation to confirm their physiological health prior to inclusion in the study. This included an assessment of vital signs and complete haematological and biochemical profiles. The mares were confirmed to be neither pregnant nor in oestrus during the sampling period.

Training protocol. All exercise sessions began at 08:00 and lasted one hour. They followed a standardised sequence designed to simulate moderate workload conditions. The sequence was as follows: walking (5 minutes), trotting (15 minutes), walking (10 minutes), trotting (10 minutes), walking (5 minutes), galloping (5 minutes), walking (10 minutes). This protocol was applied consistently across seasons and subjects to ensure consistent workloads and enable valid comparisons of seasonal and post-exercise metabolic changes.

Blood sample collection. Blood samples were drawn from the ponies' jugular veins while they were at rest. Pre-exercise samples were collected between 07:30 and 08:00, while the horses were at rest in their stalls. Post-exercise samples were obtained immediately after physical activity, between 9:00 and 9:30 am. Each horse was sampled twice: before and after the training protocol. Blood was collected into tubes containing 3.8% sodium citrate as an anticoagulant.

Assays for measuring the resistance of erythrocytes to acid-induced haemolysis. The resistance of erythrocytes to acid-induced haemolysis was assessed using the method of Gitelzon I. I. and Terskov I. A. (1955). Briefly, the erythrocytes were washed three times with isotonic saline (0.9% NaCl), after which a 2% suspension of erythrocytes was prepared. For the haemolysis assay, this suspension was mixed with hydrochloric acid (0.2 N HCl) and incubated at 37 °C. Samples were taken at 30-second intervals and the absorbance of each sample was measured spectrophotometrically at 540 nm to quantify the released haemoglobin. Complete haemolysis of the control samples was used as a reference point to calculate the percentage haemolysis. The rate of haemolysis reflects the stability of the erythrocyte membrane under acidic conditions. Slower haemolysis indicates greater membrane resistance, whereas faster haemolysis suggests increased fragility. All experimental conditions, including HCl concentration, incubation time and temperature, were carefully standardised to ensure reproducibility.

Statistical analysis. All data were analysed using Statistica 13.3 (TIBCO Software Inc., Palo Alto, California, USA). The degree of erythrocyte haemolysis was recorded at 0.5-minute intervals over a 32.5-minute period for four experimental groups: stallions before and after exercise, and mares before and after exercise. Descriptive statistics were calculated for each group, including the mean percentage of haemolysed erythrocytes, the standard deviation (SD) and the time to half-lysis (t_{50}), which is defined as the time at which 50% maximal haemolysis is achieved. The normality of the data distribution was assessed using the Shapiro-Wilk test.

To evaluate the effects of sex and exercise on haemolysis kinetics, a two-way repeated measures ANOVA was performed, with time as the within-subject factor, and sex (stallion vs mare) and exercise condition (before vs after) as the between-subject factors. Post-hoc comparisons were conducted using the Tukey multiple comparison test to identify significant differences between groups at specific time points. A p-value of less than 0.05 was considered statistically significant. Additionally, the area under the curve (AUC) was calculated for each haemolysis profile to quantify the overall extent of erythrocyte lysis over time. Differences in AUC values between groups were analysed using one-way ANOVA followed by Bonferroni correction. Graphical representations of haemolysis curves were generated to illustrate group differences and time-dependent changes in erythrocyte membrane stability (Stanisz A., 2006, 2007).

Results. We investigated the resistance of red blood cells to 0.2 N HCl in sport horses. Figure 1 shows the haemolysis curve demonstrating erythrocyte resistance to 0.2 N HCl in sporting stallions and mares during exercise.

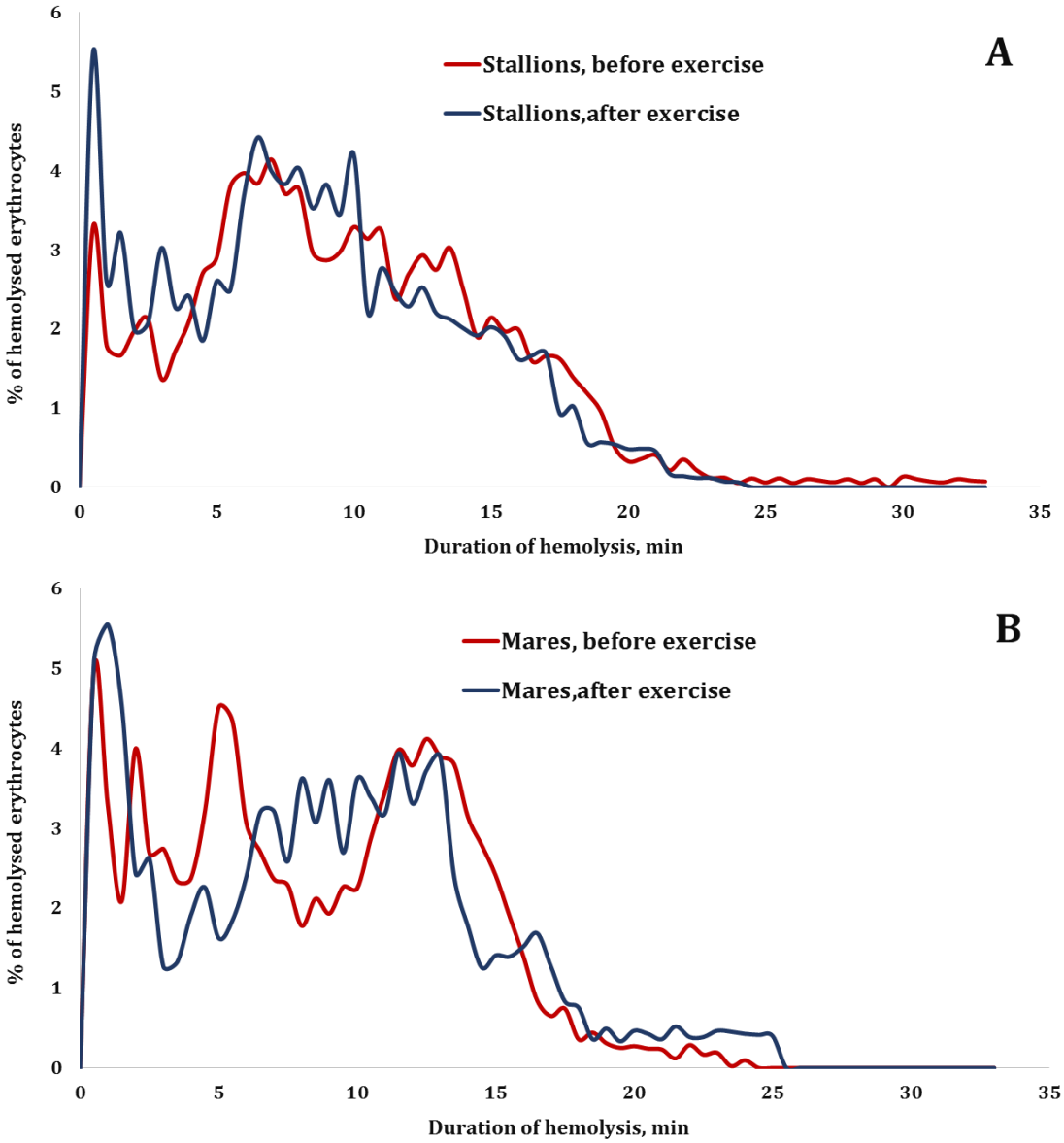

Fig. 1. **The haemolysis curve showing erythrocyte resistance to 0.2 N HCl in sport stallions (A) and mares (B) during exercise.**
The x-axis shows exposure time in minutes.
The y-axis shows the degree of erythrocyte lysis.
The results are expressed as the mean (n = 10 in each group).

The obtained data illustrate time-dependent haemolysis of erythrocytes in both stallions and mares, before and after exercise, when exposed to 0.2 N hydrochloric acid. The optical density (absorbance) values correspond to the degree of haemoglobin release during erythrocyte lysis, thus reflecting the red blood cells' resistance to acidic stress.

At the initial time point (0 minutes), no haemolysis was observed in any group (absorbance = 0). Within the first minute, however, a rapid increase in optical density occurred, indicating the onset of erythrocyte destruction. The highest values were generally recorded between 0.5 and 7 minutes, after which there was a gradual decline, suggesting progressive cell lysis and a subsequent decrease in the number of intact erythrocytes.

In stallions, the erythrocyte resistance pattern showed greater absorbance peaks following exercise than before exercise, especially during the first five minutes of acid exposure (e.g. 5.49 at 0.5 minutes after exercise versus 3.29 beforehand) (Fig. 1A). This suggests that physical exertion temporarily reduces erythrocyte stability, making the cells more susceptible to acid-induced haemolysis.

A similar pattern was observed in mares: absorbance values after exercise were consistently higher in the early phase of haemolysis (up to ~10 minutes), implying decreased membrane resistance post-exercise. However, after approximately 12-15 minutes, the curves for mares before and after exercise converged, indicating that most erythrocytes had lysed and the difference between the two groups had diminished (Fig. 1B). After 20 minutes, the absorbance values in all groups dropped markedly, approaching zero after ~25-30 minutes. This corresponds to the complete lysis of erythrocytes in 0.2 N HCl.

Statistical analysis using repeated measures ANOVA revealed a significant main effect of exercise ($p < 0.01$), confirming that physical exertion increased erythrocyte haemolysis over time. The main effect of sex was marginally significant ($p = 0.06$), suggesting a tendency towards greater membrane stability in stallions. The interaction between time and exercise was highly significant ($p < 0.001$), reflecting accelerated haemolysis kinetics post-exercise. No significant interaction was found between time and sex, suggesting that both stallions and mares followed similar haemolysis trajectories.

Post hoc comparisons using Tukey's test confirmed significant differences between the pre- and post-exercise conditions within each sex ($p < 0.05$). However, differences between stallions and mares post-exercise were not statistically significant ($p > 0.1$). These findings support the hypothesis that exercise transiently compromises erythrocyte membrane integrity, leading to increased haemolysis, regardless of sex.

The results indicate that erythrocyte resistance to 0.2 N HCl was slightly higher in stallions than in mares under resting conditions. Both stallions and mares experienced decreased erythrocyte stability due to physical exercise, resulting in faster and more intense haemolysis. The time course of haemolysis suggests a biphasic pattern: a rapid initial phase (0.5-10 minutes) followed by a slower decline (10-25 minutes) until complete lysis occurs. In conclusion, exercise significantly alters the stability of red blood cell membranes in horses, making them more susceptible to acid-induced haemolysis. Although stallions may have slightly more resilient erythrocytes at rest, both sexes experience comparable membrane destabilisation after exercise.

Discussion. The present findings demonstrate that erythrocyte resistance to acidic stress in horses, as measured by exposure to 0.2 N HCl, is influenced by both physiological status (before versus after exercise) and sex (stallions versus mares). The time-dependent progression of haemolysis observed reflects the gradual breakdown of erythrocyte membranes in acidic conditions, with changes in optical density corresponding to the release of haemoglobin into the medium. This method is a reliable, albeit classical, indicator of the mechanical and chemical stability of erythrocyte membranes, and is often used to evaluate the impact of oxidative stress, physical activity and metabolic state on red blood cell integrity. Importantly, using HCl provides a standardised, reproducible model for evaluating membrane fragility and allows comparative analysis across experimental groups. However, it should be noted that this approach does not consider the effect of plasma antioxidant capacity on erythrocyte resilience in stressful situations.

In their study, Pakula P. D. et al. (2023) demonstrated that intense physical exercise in endurance horses causes pronounced haemolysis, the severity of which correlates with the speed and distance of the race. Biochemical and metabolomic analyses

revealed significant increases in haemolysis markers after the race, particularly in horses eliminated for metabolic reasons. These findings confirm that physiological overload leads to red blood cell damage driven by mechanical and metabolic factors. In the context of our results, they corroborate the hypothesis that exercise reduces the resistance of red blood cell membranes to acid stress, thereby accelerating the haemolytic process. This supports the broader concept that erythrocyte integrity is compromised by both mechanical strain and metabolic shifts associated with prolonged exertion.

The haemolysis kinetics observed in this study were biphasic. The initial phase, which occurred within the first 0.5-10 minutes, was characterised by a rapid increase in optical density, indicating an accelerated rate of erythrocyte membrane disruption. This phase likely reflects erythrocytes with lower membrane resistance undergoing lysis almost immediately after acid exposure. The subsequent, slower decline phase (10-25 minutes) represents the progressive lysis of more resistant cells and the approach to complete haemolysis. This was achieved after approximately 25-30 minutes in all groups. A similar biphasic pattern has been reported in previous studies examining osmotic and chemical haemolysis in equine erythrocytes, and is generally considered to be indicative of heterogeneity within the erythrocyte population with regard to membrane composition and antioxidant defence mechanisms (Muñoz A. et al. 2008). This heterogeneity may be due to differences in the age of red blood cells, the composition of their cell membranes, and their exposure to oxidative agents during training.

A study by Muñoz et al. (2008) demonstrated that physical exercise in horses causes changes in erythrocyte volumetric indices, including packed cell volume (PCV), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC). These changes reflect the dynamic alterations in red blood cell morphology and function that occur during exertion. The increase in PCV observed across all exercise types indicates haemoconcentration, primarily due to splenic contraction and fluid shifts associated with dehydration. While changes in MCV and MCHC varied between horse groups, the results showed that erythrocyte size was partly influenced by plasma sodium concentration. This suggests that red cell volume is modulated by an osmotic component. Importantly, the study concluded that these erythrocyte responses were not directly correlated with overall hydration or electrolyte balance, but rather represented adaptive adjustments to exercise-induced stress. In light of these findings, it is reasonable to suggest that exercise-induced physiological changes, such as osmotic fluctuations, acidosis and mechanical strain, can temporarily modify erythrocyte stability and increase susceptibility to haemolysis, as was observed in our study under acid-induced stress conditions. Further investigation into the relationship between electrolyte shifts and membrane permeability could improve our understanding of these adaptive mechanisms.

Hanzawa K. et al. (1999) examined how exercise influences erythrocyte osmotic fragility (EOF) in horses, as well as its relationship with blood pH, temperature and other physiological factors. Red blood cell haemolysis rates were strongly correlated with blood pH and temperature: lower pH increased fragility, while higher temperature decreased it. Heart rate and packed cell volume had a relatively minor effect on changes in OFE. The findings suggest that changes in blood flow and splenic erythrocyte release induced by exercise have little impact on OFE, while pH and temperature are key modulators. Overall, blood pH and temperature play central roles in determining erythrocyte resistance to osmotic stress during exercise. These parameters should therefore be monitored closely in future studies that assess erythrocyte vulnerability under different environmental and training conditions.

Building on this, a comparison of stallions and mares at rest revealed slightly greater erythrocyte stability in stallions, as indicated by lower absorbance peaks and a

longer half-lysis time (t_{50} = 15.0 minutes in stallions versus 14.5 minutes in mares). This difference may be associated with hormonal or metabolic variations that influence membrane lipid composition and redox balance. Testosterone has been reported to modulate erythropoiesis and red cell turnover, which could contribute to the enhanced mechanical stability of erythrocyte membranes in males (Fried W. et al., 1964; Schooley J. C., 1966; Bachman, E. et al., 2014). Conversely, oestrogen-related differences in membrane fluidity and antioxidant enzyme activity could make mares' erythrocytes slightly more susceptible to oxidative or chemical damage. Also, estrogens are known to improve membrane fluidity and increase the activity of antioxidant enzymes like glutathione peroxidase (GSH-Px), which protects against oxidative stress (Massafra C. et al., 2002; Tsuda K. et al., 2002). However, further studies incorporating hormonal profiling and membrane lipidomics would be necessary to confirm these mechanistic links. Nevertheless, the interaction between sex hormones and erythrocyte membrane dynamics in equine athletes is not well understood and requires targeted investigation using advanced lipidomic and proteomic techniques.

A study by Cywińska A. et al. (2011) demonstrated that exercise-induced intravascular haemolysis occurs in racehorses, but that it exhibits distinct gender-dependent patterns. Following routine training sessions, mares exhibited increased plasma haemoglobin and decreased serum haptoglobin levels, indicating transient haemolysis. In contrast, stallions did not display such changes. Mares also had higher baseline haptoglobin concentrations, suggesting a greater capacity to adapt to the oxidative and mechanical stress associated with exercise. The authors concluded that exercise-induced haemolysis in horses is of limited clinical relevance, as it is mild and transient with no cumulative effects. These findings support the view that haemolytic responses to physical exertion depend on physiological adaptation and gender-specific factors, which may influence erythrocyte membrane stability and antioxidant defences. In the context of our results, they highlight that susceptibility to acid-induced erythrocyte damage may also vary depending on the animal's adaptive status and its individual physiological characteristics. This highlights the importance of incorporating sex-based physiological profiling into experimental designs that assess erythrocyte fragility and stress responses.

Andriichuk A. and Tkachenko H. (2017) examined the effects of moderate-intensity exercise on haematological, biochemical and oxidative parameters in Holsteiner stallions and mares. Both sexes exhibited adaptive haematological responses to exercise, though the magnitude of the changes differed between the two sexes. Stallions showed significant increases in erythrocyte count, haemoglobin concentration, haematocrit, leukocytes and granulocytes after exercise, along with elevated aspartate aminotransferase (AST) activity, which suggests higher metabolic and oxidative stress. In contrast, mares demonstrated a reduction in platelet volume after exercise. Despite these differences, the acidic resistance of erythrocytes (tested in 0.1 M HCl) remained similar in both groups and there were no statistically significant changes in the percentage of haemolysed cells before or after exercise. These findings suggest that gender-related physiological responses are primarily expressed in haematological and enzymatic parameters under moderate workloads, while erythrocyte membrane stability remains largely unaffected. However, more intense or prolonged exercise could reveal differences in membrane resilience that are not apparent under moderate conditions. Our previous findings suggest that the biomarkers of aerobic and anaerobic metabolism are largely independent of sex, indicating the presence of stable adaptive mechanisms that help ponies to maintain blood homeostasis throughout the year (Tkaczenko H. et al., 2024).

Exercise had a significant impact on erythrocyte stability in both sexes (Fig. 1). Immediately after physical exertion, the acid haemolysis curves shifted upwards, indicating higher absorbance peaks in the initial exposure period (0.5-5 minutes). This indicates an increased rate of haemoglobin release, which corresponds to reduced erythrocyte resistance to 0.2 N HCl. While the mean optical density and area under the curve (AUC) values remained consistent before and after exercise, the earlier onset of lysis (t_{50} decreased to 11.0 minutes in stallions and 13.0 minutes in mares) suggests a transient decrease in erythrocyte membrane integrity. This reduction in resistance following exercise can be attributed to various physiological mechanisms, such as an elevated body temperature, an increased plasma lactate concentration, acidosis and an enhanced generation of reactive oxygen species (ROS) during muscular activity (Wang F. et al., 2021; Powers S. K. et al., 2024). These factors may alter lipid-protein interactions within the erythrocyte membrane, promote the peroxidation of unsaturated phospholipids and lead to reversible changes in membrane permeability (Andriichuk A. et al., 2014). Such changes may also be influenced by the duration and intensity of exercise, as well as the horse's individual antioxidant capacity. These factors should be considered in future studies. These alterations are usually short-lived and reversible, consistent with the rapid recovery of erythrocyte parameters observed in post-exercise studies.

Andriichuk A., Tkachenko H. and Kurhaluk N. (2014) investigated the impact of gender on oxidative stress biomarkers, antioxidant responses and erythrocyte stability in well-trained sport horses. The results revealed that both stallions and mares experienced oxidative stress following exercise; however, the antioxidant defence mechanisms were gender-dependent. Stallions exhibited reduced lipid peroxidation and increased glutathione reductase activity, suggesting an improved redox balance and protection against oxidative damage. Mares, on the other hand, demonstrated higher superoxide dismutase activity after exercise, reflecting a compensatory antioxidant response. Notably, no significant gender differences were observed in erythrocyte osmotic resistance or the degree of haemolysis before or after exercise, suggesting that training adaptation mitigates exercise-induced erythrocyte damage. These findings emphasise that, while oxidative stress responses differ between sexes, training status plays a crucial role in maintaining erythrocyte membrane integrity during physical exertion. This emphasises the importance of long-term conditioning in reducing cellular vulnerability, regardless of gender.

The convergence of haemolysis curves between 12 and 15 minutes post-exposure indicates that the differences between pre- and post-exercise erythrocytes are negligible once the more fragile subpopulation has been lysed. This indicates that exercise-induced membrane destabilisation primarily affects the most susceptible erythrocyte fraction, while more resilient cells remain relatively resistant to acid stress. The total duration required for complete lysis remained consistent across all groups, suggesting that exercise does not permanently compromise the structural integrity of erythrocytes, but rather temporarily shifts the balance towards greater fragility. This transient effect may play a role in the physiological process of removing damaged or senescent erythrocytes from circulation (Lippi G. and Sanchis-Gomar F., 2019). Such selective clearance may help to maintain optimal blood rheology and oxygen transport efficiency during repeated periods of exertion.

The observed results are consistent with previous reports describing a decrease in erythrocyte osmotic resistance and an increase in oxidative haemolysis following physical exercise in horses and other athletic animals (Bazzano M. et al., 2015). This phenomenon may be physiologically significant in terms of adaptive erythrocyte turnover: mechanical and oxidative stress during exercise damages older or less stable red blood cells more

readily, prompting their removal from circulation and stimulating erythropoiesis. This process replaces senescent cells with younger, more deformable erythrocytes that are better suited to oxygen delivery during repeated exertion (Schmidt W. et al., 1988; Smith J. A., 1995; Mairbäurl H., 2013). This adaptive mechanism highlights the dynamic nature of erythrocyte populations in athletic species and the importance of red cell resilience in maintaining performance and oxygen transport efficiency. It also suggests that erythrocyte fragility could be used as a biomarker to indicate training load and recovery status, which could be useful in equine sports medicine.

Bazzano M. et al. (2015) investigated the effects of dietary polyunsaturated fatty acid (PUFA) supplementation on erythrocyte osmotic fragility (EOF) and related blood parameters in show jumping horses. PUFA-supplemented horses exhibited a smaller increase in blood lactate, haematocrit, red blood cell count and haemoglobin following intense jumping exercise compared to the control group. Furthermore, PUFA supplementation was found to reduce EOF, suggesting enhanced erythrocyte resistance to the osmotic stress induced by exercise. These results suggest that PUFA supplementation could enhance cellular membrane stability and improve the overall physiological performance of high-level athletic horses. EOF assessment was confirmed as a useful indicator of exercise-induced stress and athletic capacity. Further research is needed to explore the long-term effects of PUFA supplementation on erythrocyte turnover and oxidative resilience in horses competing in different disciplines.

Andriichuk A. et al. (2016) conducted a study to evaluate the effects of moderate-intensity exercise on oxidative stress, antioxidant enzyme activity and erythrocyte osmotic resistance in well-trained Ukrainian Warmblood and Holsteiner horses. Exercise increased erythrocyte count, haemoglobin and haematocrit, while regular training enhanced antioxidant enzyme activity and reduced oxidative stress. Following exercise, lipid peroxidation and protein oxidation in erythrocytes decreased; however, breed-specific differences in catalase, glutathione reductase and superoxide dismutase were observed. A significant increase in erythrocyte haemolysis after exercise indicated impairment of cell stability related to oxidative stress. These results imply that monitoring oxidative stress and antioxidant biomarkers could offer valuable insights into the fitness, health, and performance of equine athletes. Training protocols and recovery strategies could be further refined by incorporating breed-specific physiological profiles.

In summary, exposing equine erythrocytes to 0.2 N HCl revealed clear differences in membrane stability related to exercise and sex. Physical activity was found to transiently decrease erythrocyte resistance to acid-induced lysis, reflecting post-exercise oxidative and metabolic stress. Under resting conditions, stallions showed marginally higher resistance than mares, suggesting sex-related physiological differences in membrane composition and redox homeostasis. These findings further our understanding of the behaviour of erythrocytes under stressful conditions and could inform the evaluation of the physiological condition, training adaptation and recovery status of horses in sporting and endurance disciplines. Thus, the evidence suggests that erythrocyte fragility testing can be used as an additional tool for monitoring performance and managing equine athletes.

Conclusions. This study shows that the resistance of red blood cells to acid-induced haemolysis in horses is affected by exercise and sex. Physical exertion temporarily reduces erythrocyte stability, primarily affecting the most vulnerable subpopulation. This is likely due to the oxidative stress, acidosis and metabolic strain induced by exercise. Under resting conditions, stallions generally exhibited slightly higher erythrocyte resistance than mares, suggesting sex-related differences in membrane composition and redox homeostasis. However, these differences were temporary and

overall membrane integrity was restored after exercise, indicating the resilience of erythrocytes to physiological stress in well-trained horses.

Our findings emphasise the importance of considering physiological status and sex when evaluating erythrocyte stability, and highlight acid-induced haemolysis as a valuable tool for assessing red blood cell integrity under stress. Furthermore, the study reinforces the concept that regular training and dietary interventions can modulate erythrocyte resistance and support optimal oxygen transport during exercise. These insights could inform targeted strategies for monitoring fitness, optimising recovery and enhancing performance in equine athletes.

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