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Assessment of Serum Heat Shock Protein (HSP70) and Cortisol Concentration Change in Horses before and after an Acute Exercise

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ABSTRACT

This study was conducted to secure basic data for improving the welfare of domestic breeding horses by measuring the accumulation of heart rate and lactic acid before and after exercise and measuring the concentration of Cortisol and HSP70 as indicators for horse stress. For three sessions before exercise, immediately after exercise, and 30 min after exercise, 15 Thoroughbred racing horses and 15 riding horses in domestic breeding were designated, and lactate was measured in plasma using a poly equine health check Polar H10 heart rate meter. In addition, the concentrations of HSP70 and Cortisol, which can be taken as values of stress in three sessions, were analyzed. The levels of HSP70 were measured as 29.43±5.72pg/mL before exercise, 89.74±23.02pg/mL after exercise, 32.19±6.57pg/mL after 30 min of exercise, 41.25±8.26pg/mL before exercise, and 114.02±29pg/mL after exercise (pg/mL) after exercise. Cortisol concentrations were found to be 3.38±1.2µg/dL before exercise, 5.11±1.16µg/dL immediately after exercise, 5.58±2.15µg/dL after 30 min of exercise, 6.61±2.04µg/dL before exercise, 8.47±2.55µg/dL immediately after exercise, and 30 min after exercise. Hematology chemistry in riding horse and racing horses had some differences by items, but there was no significant difference overall. In conclusion, it has been confirmed that racing horses have higher stress from strong exercise compared to riding horses, and plans to use it to promote the welfare of domestic horses in the future.

Key words: Cortisol, Horses, Heat Shock Protein (HSP70), Serum

INTRODUCTION

Heat shock proteins (HSP70) are found in all species, from bacteria to humans (Udono and Srivastava 1993; Moseley 1998; Kregel 2002; Cosemans et al. 2022). A group of proteins produced by cells in stressful situations that protect cells from potentially fatal situations and extreme conditions, and HSPs have been found in relation to heat shock but play a role in various types of stress reactions, including cold exposure, wound healing, and infection (Locke et al. 1990; Kültz 2005; Hartl et al. 2011; Noble and Shen 2012; Saibil 2013; Sheykhbahaei et al. 2022; Kim et al. 2023).

The number of HSP increases due to the folding, incorrect folding or aggregation of proteins, but other reasons include oxidative stress, malnutrition, infection of the virus, and exposure to cytokines (Garbuz et al. 2019).

Conditions that trigger cellular stress responses are reactions to physical exercise that cause HSP increases in various types of cells and tissues, including high fever, ischemia, oxidation, cytokine and muscle stress, and glucose deficiency and exercise and calorie restriction are a form of stress that increases HSP numbers (Locke et al. 1990).

Studies have shown that exercise is associated with a temporary increase in HSP expression in sulcur, humans, and horses, and changes in muscle load due to changes in treadmill exercise intensity in rodents were also an important component of the increase in HSP70 content (Serrano et al. 2002; Kinnunen et al. 2005). In addition, the relationship between stress response and physical exercise in humans has been studied, but studies on stress response and expression of HSP according to horse exercise intensity are somewhat limited (Ziemann et al. 2013).

Currently, as the domestic horse industry develops, the number of racehorses and horseback riding is increasing a lot and a total of 34,793 horses are registered as of May 2022 in Korea. There are 3,436 horses for racing, 13,518 horses for riding, 3,252 horses for breeding, 11,382 horses for recreation, testing, and for ornamental purpose. Of the 13,581 horses registered with the Korea Racing Authority, 5,130 are using the retired racehorse, Thoroughbred horses.

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It is good for ordinary people who enjoy horseback riding to ride the Warmblood, which is gentle in nature and is widely used as a riding horse, but the price of horses cannot be raised in general horseback riding grounds due to the characteristics of being imported. In addition, there is a lot of support for domestic horses due to the policy to encourage domestic horses, but there are many difficulties due to the burden of raising them from an early age and the breeding environment.

Therefore, the retired racehorse, Thoroughbred horse, is converted and provided to horseback riders at the horseback riding club, and Thoroughbred horse has a running habit because it is used for racing at the racetrack, so there are many elements of risk.

Racehorses do aerobic exercise in addition to racing, but they do strong aerobic exercise with a speed of 13-15 sec in furlong time, and horseback riding generally does not do fast and strong exercise compared to racehorses (Mercier and Aftalion 2020).

In addition, due to the nature of horse racing, the reality is that if you win, you will compete because of the prize money system, so you will exercise more severely.

In study, HSP72 and HSP70 were expressed in response to stress caused by acute exercise in Standardbred horse, and based on the study, it was hypothesized that racing horses would have higher stress responses than riding horses (Avenatti et al. 2018; Khummuang et al. 2020).

In addition, in the case of riding horse, it has been studied that the higher the exercise intensity, the higher the stress level and the higher the dangerous behavior change of the horse. As times change, the importance of animal welfare is emphasized and there are some views that horse racing and horseback riding are also animal abuse.

In this study, the accumulation of heart rate and lactic acid before and after exercise of racing horses and riding horses raised in Korea was used as an indicator of exercise volume, and the concentration of HSP70 and Cortisol were measured to secure basic data for improving the welfare of domestic horses.

MATERIALS AND METHODS

Ethical Approval

The protocol and conduct of this study were approved by the Kyungpook National University of Animal Ethics Committee, Republic of Korea (KNU2019-0091). It also complied with the AALAC regulations (Newcomer and McGlone 2015).

Animals

The horses used in this study were studied with a total of 30 horses, 15 racehorses used at the Busan Gyeongnam Horse Racing Park of the Korea Racing Authority (2 to 6 years old, 7 mares, 4 stallions, and 4 gelding, average weight of 480±50 kg, all Thoroughbred horse) and 15 riding horses used in horseback riding club (4 to 15 years old, 6 mares, 3 stallions, 6 gelding, average weight of 500±50kg, 6 Warmblood horses and 9 Thoroughbred horses).

The racing horses momentum proceeded as follows at a speed that was the most similar to that of the race. A total of 35 min of exercise was performed in the order of 5 min walk (1.6-2 m/s), 10 min of trot (3.3-3.5 m/s), 1400 m gallop (10.7-11 m/s) (approximately 2 min), 10 min of trot (3.3-3.5 m/s) and 5 min of walk (1.6 m/s) of walking machine.

A treadmill was used to match a certain amount of exercise for riding horses. A total of 40 min of exercise was performed in the order of 5 min of walk (1.6 m/s), 10 min of trot (3.3 m/s), 10 min of canter (6.1 m/s), 10 min of trot (3.3 m/s), and 5 min of walk (1.6 m/s).

All participating horses were individually managed in the horse stable and the composition of hay and thick feed was supplied according to the energy required per day according to the momentum, and the water was freely and automatically supplied.

Heart Rate

The maximum heart rate was measured before exercise (rest period), immediately after exercise, and 30 min after exercise using a pulse measurement machine (Polar equine health check, Polar H10 heart rate sensor, Polar Electro Oy, Kempele, Finland).

Lactic Acid

Lactic acid measurements were made on plasma separated by a centrifuge (1 min at 5000 RPM) after blood was collected from the horse's jugular vein before, immediately after exercise, and 30 min after exercise.

Using a portable lactic acid analyzer (Accutrend plus system-Roche Diagnostics GmbH, Mannheim, Germany), it was analyzed according to the manufacturer's manual in an environment between 5 and 40°C.

HSP70 Analysis

HSP70 analysis of horses was performed by centrifugation (4°C, 1,000 g, 15min) of blood collected from the horse's jugular vein before, immediately after exercise, and 30min after exercise, and then serum was used for the experiment. The HSP70 was analyzed using the ELISA HSP70 high sensitivity EIA kit (Enzo Life Sciences, Farmingdale, NY, USA) at 450nm in wavelength, 9 nm in bandwidth, and 23.5°C in temperature, and then the concentration was calculated.

Cortisol Concentration

Blood was refrigerated before the start of the test, and the cortisol concentration was also analyzed as serum, and centrifugal was performed at 3000 RPM and RT for 7 min during serum separation. Cortisol was performed by IMMULITE 2000 Immunoassay System (Siemens Healthcare GmbH, Erlangen, Germany).

Hematology Chemistry

Blood was stored in the refrigerator before the test, and whole blood was used for the test, and no special treatment process was performed before the test. In the case of the CBC test, it was performed with an XN-V device from Sysmex (Sysmex, Kobe, Japan). Before the test, the blood was refrigerated and serum was used, and during serum separation, it was centrifuged at 3000 RPM and RT for 7min. Reagents were used as they were, compatible reagents provided by Mindray, and 20 biochemical tests were performed with Mindray's BS-490 device (Mindray, China), and electrolyte test was performed with Medica's Easy Electrolytes device (Medica, USA).

RESULTS

Heart Rate and Lactic Acid Concentration

The heart rate and lactic acid measurement results are shown in Table 1. Heart rate was measured before exercise (53±8 and 48±8beats/min), immediately after exercise (194±28 and 132±22beats/min), and 30 min after exercise (59±6 and 53±7beats/min) in racing horses and riding horses, respectively.

Lactic acid was measured before exercise (1.2±0.3mmol/L), immediately after exercise (19.1±6mmol/L), and after 30 min of exercise (2.8±1.9mmol/L) for racing horses, but it could not be measured outside the measurement range (0.2-0.8mmol/L) for both riding horses. The lactic acid concentration of racing horses was measured to be 0.9±0.6mmol/L before exercise, but it was significantly higher immediately after exercise and higher after 30 min than before exercise.

Both racing horses and riding horses had an increased heart rate compared to before exercise, and the maximum heart rate was 194±28beats/min for racing horses and 132±22beats/min for riding horses. The results of measuring the heart rate after 30 min of exercise was still higher compared to before exercise.

For both heart rate and lactic acid, the number of racing horses was generally higher than that of riding horses before exercise.

Table 1: Heart rate (HR) and lactic acid concentration (PLa) in three exercise sessions of riding horses (A) and racing horses (B)

Sessions		Baseline	Post-exercise	30 min post- exercise
HR (beats/min)	A	48±8	132±22	53±7
	B	53±8	194±28	59±6
PLa (mmol/L)	A	LO	LO	LO
	B	1.2±0.3	19.1±6	2.8±1.9

Values are Mean±SD. LO means a low value outside the range of the machine measurement values (0.2 to 0.8mmol/L).

HSP70 Analysis

The results of the serum HSP70 analysis are shown in Table 2. It was measured before exercise (29.43±5.72 and 41.25±8.26pg/mL), immediately after exercise (89.74±23.02 and 114.02±29pg/mL), and 30 min after exercise (32.19±6.57 and 54.40±12.33pg/mL) in riding horses and racing horses, respectively. Racing horses were higher than riding horses, and the concentration after exercise was significantly higher than before exercise. However, it did not go down to the HSP 70 concentration before exercise until 30 min after exercise.

Table 2: HSP 70 concentration in three sessions before, after and after 30 min of exercise in riding horse (A) and racing horse (B)

Session		Baseline	Post-exercise	30 min post- exercise
HSP70	A	29.43±5.72	89.74±23.02	32.19±6.57
(pg/mL)	B	41.25±8.26	114.02±29	54.40±12.33

Values are Mean±SD.

Cortisol Concentration

The results of the cortisol concentration measurement are shown in Table 3. It was measured before exercise (3.38±1.2 and 6.61±2.04µg/dL), immediately after exercise (5.11±1.16 and 8.47±2.55µg/dL), and 30 min after exercise (5.58±2.15 and 9.22±1.95µg/dL), respectively, in riding horses and racing horses. The cortisol level was significantly higher immediately after exercise and 30 min after exercise than before exercise, and the cortisol concentration did not decrease even after 30 min of exercise, but rather higher than right after exercise. And in all three sessions, racing horses were generally measured higher than riding horses.

Hematology Chemistry

The results of the hematology chemistry measurement are shown in Fig. 1 and Tables 4~5. The neutrophil (NE) count ranged between 50.9±3.4% in the riding horse and 75.9±6.2% in the racing horse. These hematology chemistry in riding horse and racing horses had some differences by items, but there was no significant difference overall.

Table 3: Cortisol concentration in three sessions before, after, and after 30 min of exercise in riding horse (A) and racing horse (B)

Session		Baseline	Post-exercise	30 min post- exercise
Cortisol	A	3.38±1.2	5.11±1.16	5.58±2.15
(µg/dL)	B	6.61±2.04	8.47±2.55	9.22±1.95

Values are Mean±SD.

DISCUSSION

In this study, as in other studies (Khummuang et al. 2020), there was a significant difference in the concentration of HSP70 before and after exercise in horses, and after exercise increased compared to before exercise. In addition, the stronger the intensity of exercise, the more pronounced the increase in cortisol and HSP70. Studies on the increase in HSP70 in exercise intensity have been shown to induce inflammatory responses such as post-exercise stress, high fever, metabolic disorders, and hormonal changes in humans (Noble et al. 2008; Ziemann et al. 2013). It seems necessary to further study how long it takes after exercise to return the concentration of HSP and cortisol to the pre-exercise state.

There have been studies in which the lactic acid concentration increases high when the heart rate reaches 200 (beats/min) or higher, which is close to the maximum heart rate, and there have been studies in which the lactic acid concentration decreases and recovers after 15 min of exercise (Schuback and Essén-Gustavsson 1998). Lactic acid is produced by anaerobic metabolism, the intensity of exercise is known to be closely related to muscle fatigue, correlates with heart rate, and other studies have shown that heart rate and lactic acid levels can be used as indicators of momentum (Schuback and Essén-Gustavsson 1998; Popov and Vinogradova 2012; Angélica et al. 2021).

Other studies have shown that in racehorses, stronger training associated with competition results in a more stressful response, and horses with higher levels of cortisol but better performance increase less than those with lower levels (Witkowska-Piłaszewicz et al. 2021).

In addition to racehorses, there have been studies on cortisol concentrations according to exercise changes in three groups for riding horses used daily in Jeju (Kang and Lee 2016). Also, there have been studies proving that the concentration of cortisol has increased after exercise such as jumping, cross-country and dressage.

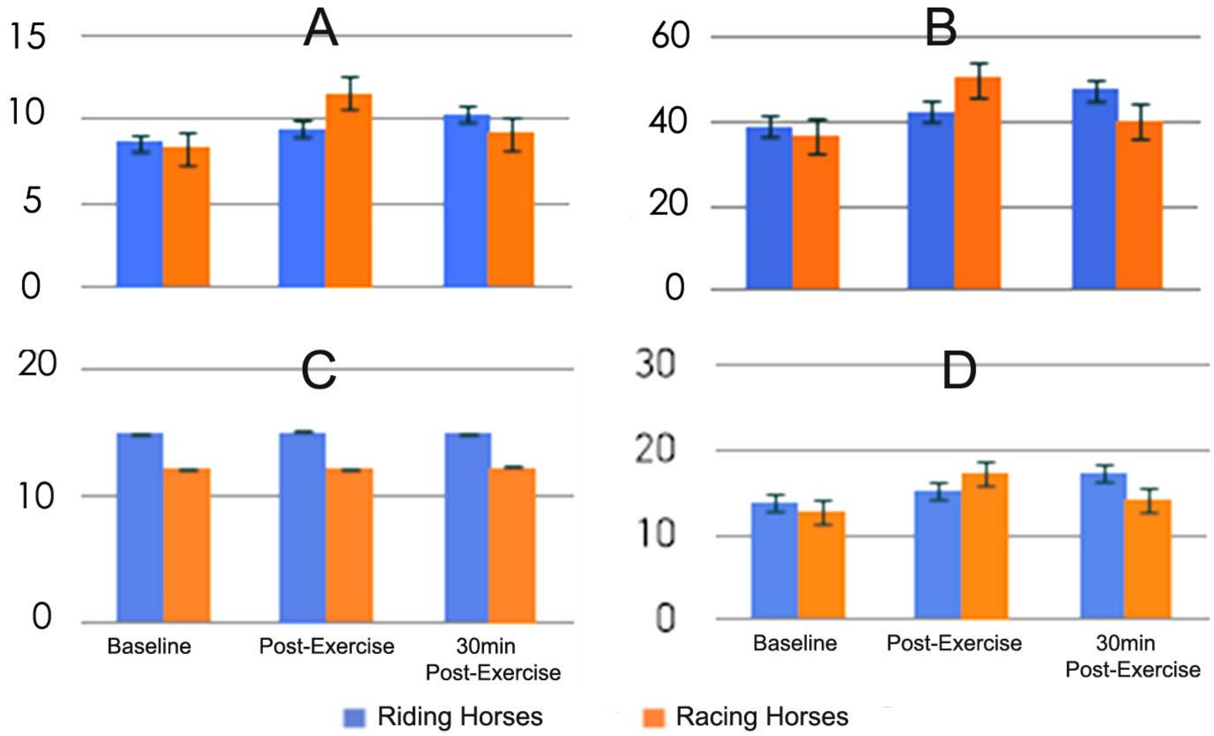

Fig. 1: The three sessions before, after exercise, and after 30 min of exercise are graphs showing A) erythrocytes level, B) hematocrit, C) urea nitrogen creatine, and D) hemoglobin comparing to riding horse and racing horse.

Table 4: Results of values for erythrometric variables and platelet count in three exercise sessions of riding horses (A) and racing horses (B)

	A			B		
	Baseline	Post-exercise	30 min post-exercise	Baseline	Post-exercise	30 min post-exercise
Total protein (g/dL)	6.3±1.2	6.4±0.8	6.7±1.4	5.4±0.6	5.8±1.1	5.4±0.9
Albumin (g/dL)	3.9±0.2	4.0±0.36	4.3±0.24	3.9±0.4	4.1±0.25	3.9±0.2
Bilirubin total (mg/dL)	1.0±0.6	1.1±0.8	1.2±0.5	3.2±0.5	3.6±0.7	3.4±0.8
Glucose (mg/dL)	93±4	84±6	85±8	131±11	102±9	98±5
BUN (mg/dL)	14.9±0.87	12.1±0.97	14.8±1.00	11.9±0.77	14.7±0.96	12.0±1.2
Creatinine (mg/dL)	1.2±0.2	1.3±0.2	1.4±0.6	1.3±0.5	1.3±0.3	1.3±0.2
RBC (M/μL)	9.45±0.81	9.17±1.66	10.26±2.1	8.27±0.92	8.65±1.74	11.54±2.36
Hb (g/dL)	14.9±2.1	13.7±1.8	16.9±2.6	12.3±1.2	13.5±1.5	17.1±2.6
HCT (%)	41.6±3.2	39.6±4.6	46.8±3.8	35.8±2.5	38.2±4.2	49.6±6.3
Uric acid (mg/dL)	0.2±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.4±0.1	0.4±0.2
AST(GOT) (U/L)	259±15	265±22	277±23	274±17	293±31	273±24
ALT(GPT) (U/L)	17±2	12±3	14±3	12±3	13±2	11±4
ALP (U/L)	167±17	160±12	166±14	157±11	168±13	155±12
Y-GTP(GGT) (U/L)	7±5	13±4	6±3	16±2	16±1	16±2
LDH (U//L)	379±24	355±23	371±17	297±18	337±14	306±13
CK(CPK) (U/L)	252±13	252±11	263±14	189±19	216±21	193±16
Cholesterol (mg/dL)	102±14	99±11	104±13	80±7	84±6	79±5
Globulin	2.4±0.2	2.4±0.4	2.4±0.6	1.5±0.3	1.7±0.7	1.5±0.7
Lipase	70±12	82±18	12±5	21±12	22±11	20±9
MCV (fL)	44.2±2.3	44.0±3.6	44.1±1.7	43.3±1.8	43.0±2.6	43.2±2.5
MCH (pg)	15.6±0.9	15.8±0.7	15.9±0.4	14.9±0.5	14.8±0.6	14.9±0.4
MCHC (g/dL)	35.3±0.8	35.8±0.5	36.1±0.7	34.4±0.2	34.5±0.7	34.6±1.1
RDW (%)	20.4±0.5	20.8±0.7	21.5±0.6	20.9±1.3	22.9±1.2	21.3±0.8
PLT (K/μL)	65±5	50±11	66±7	57±9	57±8	78±12
MPV (fL)	7.8±0.6	7.2±0.4	7.5±0.3	8.1±0.3	7.6±0.7	7.8±0.4

Table 5: Results of values for differential white blood cell count in three exercise sessions of riding horses (A) and racing horses (B)

Parameters	A			B		
	Baseline	Post-exercise	30 min post-exercise	Baseline	Post-exercise	30 min post-exercise
WBC (K/μL)	7.66±1.55	8.42±1.27	8.82±1.22	7.35±1.65	8.16±1.52	6.54±1.44
NE (%)	50.9±3.4	49.3±4.2	47.8±3.5	75.9±6.2	69.6±5.6	68.6±5.4
LY (%)	40.6±4.2	43.3±3.6	44.8±3.8	18.9±2.5	26.1±5.2	26.6±4.1
MO (%)	6.1±0.2	5.3±0.6	5.3±0.4	2.9±0.4	2.8±0.3	3.5±0.5
EO (%)	1.4±0.7	1.4±0.4	1.2±0.3	0.7±0.2	0.5±0.3	0.5±0.2
Baso (%)	1.0±0.2	0.7±0.5	0.9±0.3	1.6±0.6	1.0±0.5	0.8±0.6

Values are Mean±SD.

In this study, blood sampling was performed, but in other studies, blood sampling was performed in horse saliva without blood sampling, and eye temperature, infrared temperature, and heart rate were used (Negro et al. 2018). Also, needles used for blood collection can cause stress, and recently, pigs are being replaced by a painless injector in general needles used for vaccination in Korea and around the world. There are manuals and guidelines on horse welfare in Korea but compared to manuals and guidelines in the United Kingdom and the United States, which are advanced in horse racing, it is not enough. The International Group of Specialist Racing Veterinarians (IGSRV) has also established a racehorse welfare guideline that horse welfare should be maintained first in horse racing and should not be subordinated to competitive or commercial influences. In addition to racehorses, Federation Equestrian International (FEI)'s Code of Conduct for Horse Welfare always puts horse welfare first. In particular, racehorses and riding horses are animals used in sports with humans, so they need to pay more attention to welfare, and because they are sports partners, not just people's means of using sports, ordinary people should learn about horse health and welfare first.

Recently, the importance of animal welfare has increased and more studies have been conducted on horse stress (Valera et al. 2012; Yarnell et al. 2013; Soroko et al. 2016; Negro et al. 2018; Esteves Trindade et al. 2019; Witkowska-Piłaszewicz et al. 2021). As the Horse Industry Development Act is enacted and the domestic horse industry develops, the number of people using horse racing and horseback riding is also increasing. As racehorses that retired from being used as racehorses in Korea are highly utilized, continuous research is required in terms of safety issues and animal welfare in the future.

Conclusion

Racehorses were found to have higher stress due to strong exercise than horseback riding, and the results obtained through this study will be used to promote the welfare of horses in the future.

Author Contributions

All research protocols and animal experiments in this study designed, conducted experiments by YJ Lee, KHANDSUREN Badgar and KHOROLMAA Chimedtseren, and contributed to data acquisition. GJ Cho contributed to the interpretation of the experimental results and the writing of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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