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Research Paper

Elevation of Blood Creatine Kinase and Selected Blood Parameters after Exercise in Thoroughbred Racehorses (*Equus caballus* L.)

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ABSTRACT: *Creatine kinase, an enzyme that has essential role in the energy metabolism of organisms, was investigated in the blood of horses pre and post exercise. The experiment used four (4) normal male Thoroughbred horses weighing 500-700 kg. The blood samples were collected in the morning before subjecting the horses to different types of exercise and immediately after the physical activity. The animals performed a combination of different horse gaits which include trot and canter through lungeing and long-reining (circular bridge lungeing) as their form of exercise. Various blood tests such as the complete blood count, lipid, protein and enzyme blood tests were done. Significant elevations in the white blood cells (WBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH) and the enzyme of interest – the creatine kinase (CK) in the blood of racehorses were recorded after exercise. This study confirmed the findings of several studies on the effect of exercise on different blood parameters especially creatine kinase which can be possibly used as an index of fitness in horses.*

Keywords *- Creatine kinase, exercise, energy metabolism, Thoroughbred, blood test*

I. INTRODUCTION

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phosphocreatine kinase, is an enzyme which plays an essential role in the energy metabolism of organisms since it is involved in the synthesis and use of energy-providing molecules (Pulugurtha, 2011). A member of the phosphagen kinase family, this 82 kDa enzyme is responsible for the catalysis of the reversible transfer of phosphate from phosphocreatine to ADP, forming creatine and ATP (WebMD, 2012). The phosphocreatine efficiently supplies ATP to the skeletal muscles, heart and brain for use in physiological processes since it is used as a reservoir of phosphate containing high energy. CK plays an important role and is broadly expressed in various tissues and organs as a key regulator of ATP synthesis but it is dominantly expressed in tissues with large and fluctuating energy demands such as the skeletal muscle, smooth muscle and brain cells. Hence, it is considered a central controller of cellular energy homeostasis (Bessman et al. 1981, Saks et al. 1978, Schlattner et al. 2006a, Schlattner et al. 2006b, Walliman et al. 1992 and Walliman et al. 2007).

CK exists as four major isoenzymes characterized based on the differences in the nucleotide and amino acid sequences, tissue localization and immunogenicity (Schlattner et al., 1998). The cytosolic isoenzyme is composed of two polypeptide subunits of around 42 kDa and can either be of two types: the brain type (B) and the muscle type (M) which can form homodimers (CK-MM and CK-BB) or heterodimers (CK-MB). Skeletal muscles are composed of about 98% MM and 2% MB while 70-80% MM and 20-30% MB make up the cardiac muscles. The brain, on the other hand, has predominantly BB. Moreover, there are two specific forms of CK in the mitochondria (Mt-CK) which include the sarcomeric type expressed in cardiac and skeletal muscles and the non-sarcomeric type which is expressed in various tissues such as brain, smooth muscle and the sperm (Worthington Biochemical Corp. 2012 and Baird et al. 2011).

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Elevated blood CK levels may serve as indicators of a variety of conditions ranging from heart disease, muscular dystrophy, nerve damage to thyroid disorders and kidney malfunction. Factors that contribute to raised levels of blood CK include cell damage, muscle cell disruption, or disease which can result to leakage of CK from cells into the blood serum (Pulugurtha, 2011 and Totsuka et al., 2002 as cited by Baird et al., 2011). However, there have been reports being made on the significant relationship between muscular exercise and energy metabolism. In fact, elevated creatine kinase levels in the blood is said to be associated to muscle cell damage and disturbance following strenuous exercise (Baird et al., 2011).

The above mentioned physiological functions of creatine kinase provide detailed information on the role of CK in the production of energy in the body for the optimum functioning of body tissues and organs. Knowledge on CK and the influence of exercise and strenuous physical activity has on this enzyme may serve a very good purpose in coming up with a good management practice particularly on the aspect of giving the horses appropriate regular physical exercise for their health and wellness. Furthermore, the possibility of using CK as an index of "fitness" in horses is also being considered since studies have shown that changes in the CK levels, along with other serum enzymes –lactic dehydrogenase and aldolase - were reduced by repeated exposure to exercise (Anderson, 1975).

Thoroughbred horses require heaps of energy in order to perform well. Proper care and management should therefore be provided to the racehorses so as to keep them perform at their optimum. Hence, studies related to energy metabolism will be of significance for future management-related modifications in horse farms.

This study basically aimed to compare the pre and post-exercise whole blood components with particular emphasis on the levels of creatine kinase in the blood of Thoroughbred horses. Moreover, this study provides information on the exercise-related changes in the blood of racehorses in order to identify the blood components that are highly influenced by physical exercise.

II. MATERIALS AND METHODS

2.1 Blood Collection

Blood was drawn from four male Thoroughbred horses in the morning before and after subjecting them to physical exercise. Pre and post-exercise venous blood samples were taken using a 50 ml syringe from the Thoroughbred horses weighing 500-700 kg. In this study, only male horses were selected in order to avoid future discrepancies in the results as it is known that a number of metabolic and stress/inflammatory responses are dependent on the sex (Radom-Aizik et al., 2008). The blood sampling was carried out via venipuncture in the forearm vein and carotid artery of the racehorses before and after exercise.

The animals performed a combination of different horse gaits which include trot and canter through lungeing and long-reining (circular bridge lungeing) as their form of exercise. Generally, racehorses are subjected to exercise for 17-18 minutes per day but horses in this study were engaged into a combined 30-min exercise of trot and canter, instead.

2.2 Blood Analysis

Tubes of different sizes (2.7 ml serum-separating tubes, 8-10 ml EDTA-coated tubes and 3-4 ml tubes) were used for the inspection blood clot, clinical chemistry and general blood types, respectively. The samples were stored at low temperatures during their transport to the laboratory. The blood samples were placed in serum-separating tubes (SST), centrifuged at 2500 rpm for 15 minutes and immediately stored inside the refrigerator at -80˚C until scheduled assay. The following hematological parameters were measured in EDTA-blood using a hematology automated analyzer (Sysmex XE 2100): red blood cells (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cells (WBC) while the concentrations of other parameters such as the creatine kinase (CK) were analyzed in heparinized plasma using an automatic biochemical analyzer (ADVIA 1650, Japan). The results of the analysis of the blood taken before exercise were compared with those observed after exercise.

The experimental works were done at the Molecular Genetics Laboratory, Department of Animal Science, College of Natural Resources and Life Science, Pusan National University.

2.3 Data Analysis

Paired comparison t-test was used to test the horse blood samples taken before and after exercise for any significant difference. SAS 9.11 (Cary, USA) was used to compute for the mean and standard deviation and results were expressed in mean ± SD. Significant level of differences among the samples were determined at $p<0.05$.

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III. RESULTS AND DISCUSSION

3.1. Complete Blood Count

The mean values and paired comparison t-test of the complete blood count / full blood count of the Thoroughbred horses before and after exercise are shown in Table 1. The complete blood count gives important information about the kinds and numbers of cells in the blood. The cells that circulate in the bloodstream such as the white blood cells (leukocytes), red blood cells (erythrocytes) and the platelets (thrombocytes) were primarily analyzed and assessed in the blood of the horses pre and post exercise. Each of which performs different functions in the blood physiology as well as in the entire physiological processes of racehorses. Leukocytes protect the body against infection while the erythrocytes function in carrying oxygen from the lungs to the rest of the body. Thrombocytes, the smallest type of blood cells, function in blood clotting (WebMD, 2010).

The parameters that were considered in this test include the number of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (HCT), red blood cell indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells types (differential) such as eosinophils, neutrophils, lymphocytes, monocytes, reticulocytes, erythrocyte sedimentation rate (ESR) and blood platelet counts.

Among the different parameters being analyzed, the full blood count values obtained from the pre and post-exercise horse blood samples turned out to be statistically different in terms of WBC, Hb and MCV where there was a marked increase in the aforementioned after exercise (Figures 1a and 1b). The dark and light grey-colored bars represent the blood of the racehorses before and after exercise, respectively.

Table 1. Mean values and paired comparison t-test of the complete blood count / full blood count of the Thoroughbred horses before and after exercise

Parameters	Before	After	Paired Comparison		
	Mean± SEM	Mean± SEM	$\bar{D}$ ± SE	t	Pr> |t|
RBC(B) ($10^6/\mu\ell$)	8.19±1.21	8.90± 0.68	-0.71± 0.27	-2.59	0.0813
WBC(B)($10^3/\mu\ell$)	7.01± 1.36^{b}	8.00± 1.63^{a}	-1.00± 0.14	-6.87**	0.0063
HCT (%)	36.13± 3.40	39.50± 0.98	-3.38± 1.45	-2.33	0.1023
Hb (g/dL)	14.28± 1.41^{b}	15.80± 0.56^{a}	-1.53± 0.48	-3.20*	0.0494
MCV (fL)	44.43± 2.60	44.58± 2.93	-0.15± 0.18	-0.81	0.4765
MCH (pg)	17.55± 0.93^{b}	17.78± 0.90^{a}	-0.23± 0.02	-9.00**	0.0029
MCHC (g/dL)	39.53± 0.17	39.95± 0.59	-0.42± 0.22	-1.92	0.1505
ESR (mm/hr)	37.25± 18.66	22.50± 6.61	14.75± 8.61	1.71	0.1851
Platelet ($10^3/\mu\ell$)	74.75±41.27	92.00± 43.93	-17.25± 6.94	-2.48	0.0890
Eosinophil count ($/\mu\ell$)	370±249.40	220±115.76	150±88.22	1.70	0.1876
Reticulocyte (%)	0.06±0.02	0.08±0.02	-0.02±0.01	-1.85	0.1612
S. Neutrophil (%)	63.15±2.92	64.13±7.58	-0.98±2.68	-0.36	0.7398
Lymphocyte (%)	29.20±5.48	31.65±9.21	-2.45±2.17	-1.13	0.3401
Monocyte (%)	1.48±0.59	1.03±0.30	0.45±0.26	1.73	0.1817
Eosinophil (%)	5.93±4.95	3.00±2.17	2.93±1.57	1.86	0.1594

^{a,b,c}mean values within a row with different letter superscripts differ significantly
***p < 0.05, **p < 0.01**

Exercise can be considered a form of stress since it induces physiological "tension" on the body which results to a number of chemical (hormonal) and cellular changes apart from physical change as increased blood pressure, body temperature and oxygen intake. It is also said to induce immune-like response resulting to leukocytosis which is quantitatively similar to physiological insults to the immune system (Bhatti and Shaikh, 2007). Several studies have proven that WBCs are increased during exercise in different species including humans, dogs and horses (Bhatti and Shaikh, 2007; Rovira et al., 2008; and Nesse et al., 2002).

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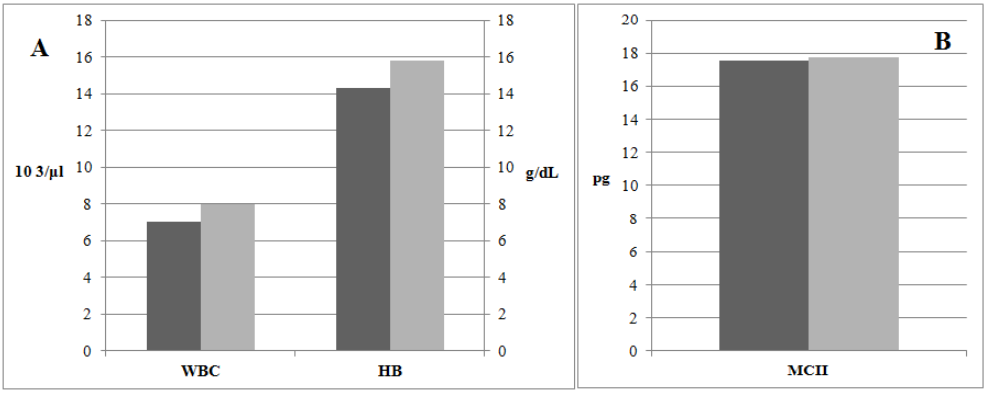

Figure 1. Significant changes in the [a] white blood cells (WBC), hemoglobin (HB) and [b] mean corpuscular hemoglobin (MCH) of the Thoroughbred racehorses before (dark grey bars) and after (light grey bars) being subjected to physical exercise.

Rovira et al. (2008) citing the works of Arokoski et al. (1993) and Munoz et al. (1999) reported that the leukocytic response to exercise is believed to be caused by mobilization of WBC from the marginal pool in response to catecholamines and has also been associated with the release of blood rich in lymphocytes from the spleen in horses. Exercise could influence immune cells so profoundly and the factors which were found out to induce physiological perturbations during physical activity apart from the factor mentioned above as studied include thermodynamic (increased temperature), physiochemical (lactic acidosis and hypoxia), hormonal (cytokine) and physical (turbulence and dynamic shear forces) which can all alter leukocyte function (Salanova B. et al., 2005; Martinez D. et al., 2006; Pedersen B.K. et al., 2002; Biffl W.L. et al., 1996 and Angelini G.D., 1990 as cited by Radom-Aizik S.R. et al., 2008).

The leukocytosis of exercise has been often compared to inflammation-like reaction. In fact, Donovan et al. (2007) and Wardyn et al. (2008) reported that a mild inflammatory response to exercise without clinical signs has been described in horses and humans, with increased levels of pro-inflammatory cytokines and leukocytosis (Rovira et al., 2008).

Hemoglobin which is a protein-based component of red blood cells with a primary function of transferring the oxygen from the lungs to the rest of the body is expressed as the amount of hemoglobin in grams per deciliter of blood. Elevated hemoglobin levels may indicate higher RBC count and is usually the result of increased RBC production as a compensatory mechanism when blood oxygen carrying capacity is compromised to meet the demand of tissues and contracted plasma volume resulting in an appearance of greater red cell volume (Sirgan, 2007). This is essentially true in this study since the RBC in the blood of horses after exercise, although not statistically significant, was higher than before engaging the horses to physical activity. Furthermore, the HCT level in the blood of horses post exercise was numerically higher which reflects higher number of RBCs. Hematocrit is the proportion, by volume of the whole blood, which is made up of red blood cells (Shiel, 2012). Changes in the HCT can be highly influenced by dehydration. Exercise can lead to slight to severe forms of dehydration depending on the intensity and duration as well as on the availability of water for rehydration. The slight numerical increase in the HCT levels can be attributed to the loss of body fluid in the racehorses during exercise which in turn has an effect on the RBC and hemoglobin levels in their blood. A similar study on horses revealed significant increase in the packed cell volume, erythrocyte count, hemoglobin concentration, mean corpuscular volume, plasma protein, total white cell count and lymphocytes in the blood samples as compared to blood samples taken before exercise (Smith, 1989).

3.2. Lipid Blood Test

The lipid blood test which was routinely performed on the blood plasma was done in order to determine the effect of exercise on the specific lipid concentrations in the blood of the horses. This blood test includes measurement of total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL), triglycerides (TG) and phospholipid. Other values which were also measured are the iron, total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC). The results of the lipid blood test of the racehorses before and after exercise turned out to be unaffected as there were no significant differences found between the values in the blood pre and post exercise (data not shown).

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3.3. Proteins and Enzymes

The blood protein test basically measures the total amount of protein in the blood. It measures the amounts of two major groups of proteins in the blood: albumin and globulin (WebMD, 2011). Blood enzyme tests, on the other hand, measure the amount of different enzymes in the blood. Enzymes play a vital role in the body as they help to control chemical reactions. There are a number of blood enzyme tests but two of the most common blood enzyme tests include troponin level and creatine kinase level tests (Lander, 2010). The blood enzyme test serves as an indicator/marker of the body's chemical processes whether or not a condition or disorder is developing within the body. Physiological processes involving heart, liver, kidney etc. utilize large amounts of enzyme materials. When cells are damaged, enzymes are released into the bloodstream. Normal readings indicate that the organs and systems in the body are functioning properly while results which do not fall within the normal profile may mean that perturbations and disturbances are present in the body (Jeanty, 2012).

Table 2. Means, standard deviations and paired comparison t-test of the blood protein and enzyme test of the Thoroughbred racehorses before and after exercise

Parameters	Before	After	Paired Comparison		
	Mean± SD	Mean± SD	$\bar{D}$ ± SE	t	Pr> |t|
T. Protein (g/dL)	5.70± 0.72	5.78± 0.85	-0.07± 0.73	-0.10	0.9246
Albumin (g/dL)	2.95± 0.39	3.00± 0.59	-0.05± 0.39	-0.13	0.9052
ALP (U/L)	126.00± 22.32	126.50± 32.51	-0.50± 18.75	-0.03	0.9804
AST (U/L)	248.00± 36.94	269.25± 63.87	-21.25± 33.95	-0.63	0.5758
ALT (U/L)	12.25± 5.25	10.00± 1.83	2.25± 2.10	1.07	0.3618
γ-GT (U/L)	4.00± 1.83	4.75± 3.30	-0.75± 1.11	-0.68	0.5472
CK (U/L)	162.75± 44.90[b]	559.75±203.58[a]	-397.00±114.36	-3.47*	0.0403
CK-MB (ng/mL)	0.41± 0.22	0.52± 0.13	-0.11± 0.07	-1.50	0.2307
LDH (U/L)	518.50±101.20	486.50±140.13	32.00± 75.62	0.42	0.7007
Amylase (U/L)	2.00± 0.00	2.50± 2.38	-0.50± 1.19	-0.42	0.7027
Lipase (U/L)	15.00± 4.69	13.50± 1.73	1.50± 2.02	0.74	0.5117

[a,b,c]mean values within a row with different letter superscripts differ significantly
***p < 0.05, **p < 0.01**

The blood proteins and enzymes that were tested in the blood of horses in this study include total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT),gamma-glutamyltranspeptidase (γ-GT), creatine kinase (CK), creatine kinase-MB (CKMB), lactate dehydrogenase (LDH), amylase and lipase. As shown in Table 2, only the CK levels were significantly elevated in the blood of the Thoroughbred horses after exercise. There were no significant differences observed in the other enzymes being tested indicative that these enzymes are not highly responsive to physical exercise.

There are similar studies on the exercise-related CK elevations in the blood of different species including humans (Anderson et al., 1975; Rovira et al., 2008 and Baird et al, 2011). Creatine kinase is a type of enzyme found in the muscles which aids in the production of phosphocreatine, a molecule utilized by the muscles for energy. CK occurs as 3 isoenzymes in skeletal muscle, cardiac muscle and nervous system and among these isoenzymes, only the skeletal muscle is thought to contribute significant elevations in the serum (VetStream, 2012). The creatine kinase is normally found in the muscles but this enzyme can leak and be present into the bloodstream brought about by injury to the membrane surrounding the muscles. Skeletal muscle injury often triggers high blood CK levels. Injuries in this muscle can be caused by muscular dystrophy, direct trauma, strenuous exercise, immobility, certain drugs, muscle injections, nerve conduction studies, muscle infection, seizures or surgery (Rienecke, 2011).It is well known that plasma activities of various enzymes such as CK and AST increase following muscle damage or strenuous exercise. AST is not muscle specific but marked elevations are usually only associated with muscle damage while CK is more muscle –specific and raised levels reflect muscle damage; peak within 2-6 hours of insult and has relatively shorter half-life of 2 hours (VetStream, 2012) although some literatures say less than an hour (Cornell University, 2012). CK has a shorter half-life and therefore will become elevated sooner and return to normal range sooner after an acute episode of muscle damage and is therefore the preferred muscle enzyme to evaluate when diagnosing and monitoring muscle recovery and considered a reliable marker of skeletal muscle injury (Wolfsheimer et al., 2005 and Siegel et al., 1980).

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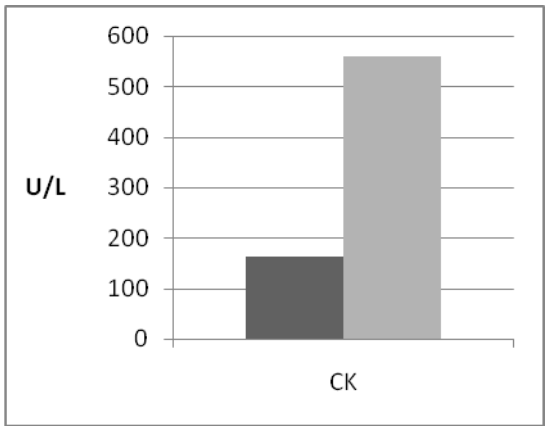

Figure 2. Significant changes in the blood creatine kinase (CK) levels of the Thoroughbred racehorses pre (dark grey bar) and post (light grey bar) exercise.

The observed significant increase in the creatine kinase levels in the blood of racehorses (see Figure 2) may indicate that the animals have experienced some degree of muscle damage or overstressing of the muscles brought about by physical exercise. Blood enzymes in endurance-trained horses were found to increase in a study conducted by Murakami et al. (1974) suggesting that the elevations in CK during exercise might indicate the severity of the exercise. It is however noteworthy to report that elevated CK levels after exercise does not necessarily mean there is a massive fibrillar disruption that took place. The enzyme activities may change with exercise due to increase in the metabolic process taking place in the muscle and transient changes in the muscle permeability without any significant fibrillar damage (Munoz et al as cited by Rovira et al., 2008). The latter may be true in this study since signs indicative of physical exhaustion or exercise intolerance and evidence of muscle disorders were not observed in the Thoroughbred horses.

IV. CONCLUSION

The results of the study showed significant elevations in the WBCs, Hb, MCH and CK in the blood of racehorses after being subjected to exercise. The study basically reinforced the findings of several studies on the effect of exercise on different blood parameters especially CK which can be possibly used as an index of fitness in horses.

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