Usefulness of Creatine Kinase Activity Determination for Assessing the Effects of Physical Effort in Horses

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ABSTRACT

Creatine kinase (CK), one of the most characteristic muscle tissue enzymes, catalyzes transfer of phosphate from phosphocreatine to adenosine 5′-diphosphate (ADP). The CK activity during exercise depends largely on the duration and intensity of exercise. The physiological range of activity of this enzyme in the blood plasma of clinically healthy horses is in the range from 90 to 275 U/L. Common phenomenon, especially in endurance horses, is the occurrence of a much larger post-exercise CK activity. However, the increase in CK activity in the blood plasma is a frequent phenomenon in endurance horses and should not be interpreted as an undesirable symptom, unless it is accompanied by clinical signs of myopathy or is still persisting over a longer period of time to increase the activity of this enzyme in the blood. It is recommended to collect blood from the horse directly before and after (up to 5 min.) exercise and 15, 30, 60 minutes or 24 hours after the exercise. Due to the various analytical methods applied and various units in which the results are presented, it seems that it would be appropriate to present variability of post-exercise CK activity in percentages, which will facilitate the assessment of this phenomenon in different individuals, often subjected to different loads. In horses especially important for diagnostic purposes is a muscle isoform (M-CK), present only in striated muscle, which probably determines to a large extent, their potential aerobic. The paper discusses creatine kinase function and usefulness for determining the activity of this enzyme during the physical effort of horses.

INTRODUCTION

Equine exercise physiology research has focused on assessing the physiological capacity and adaptability of horses to specific exercise loads (Malinowski et al., 2002; Krumrych, 2010; Janicki et al., 2013b, 2013c). Many observations have shown that creatine kinase (CK) activity in blood, is a good indicator of exercise-induced muscle cell damage (Harris et al., 1990; Janicki et al., 2013b). It is essential that horses are adequately prepared for performing often exhausting loads, because their values may approach the maximum physiological capacity of these animals, especially in the final stage of training and during competition. Post-exercise changes in the value of many blood parameters are generally very clear but they quickly subside (Zobba et al., 2011). Overexertion usually makes these changes more persistent and may be indicative of overtraining (Padalino et al., 2007). Therefore, the analysis of the blood CK activity may be useful for assessing the endurance adaptations of horses to exercise loads (Szarska, 2001).

Creatine kinase, discovered by Lohmann in 1934, is one of the most characteristic muscle tissue enzymes that catalyzes transfer of phosphate from phosphocreatine to adenosine 5′-diphosphate (Lohmann, 1934; McLeish and Kenyon, 2005; Kędzierski, 2011). A temporary small increase in serum CK activity is mainly associated with abnormal cell membrane permeability as a result of lipid peroxidation and muscle tissue hypoxia during physical effort (Nimmo and Snow, 1982). A particularly large role attributed to expression of the CKM candidate gene coding for a type of creatine kinase muscle isoenzyme, which occurs only in striated muscles and may influence the efficiency of sport horses (Gu et al., 2010; Hill et al., 2011).
Type I muscle fibres are characterized by lower activity of muscle creatine kinase compared to type II fibres, which may be advantageous to sport horses whose muscles are composed primarily of type IIA and IIB fibres (Schröder et al., 2011). The present review is focused on the usefulness of determining creatine kinase activity for assessing the horse’s adaptation to performing different exercise loads.

Role of creatine kinase in muscles: The main source of energy in the muscle cell is adenosin5′-triphosphate (ATP), the concentration of which is relatively low (2-5 mM), which allows muscles to contract for just several seconds. This being the case, additional metabolic processes have to take place in the activated muscle cell to maintain ATP homeostasis. It is determined by large reserves of phosphocreatine (20-35 mM), as a result of which ATP is regenerated in the cell after a reversible reaction catalyzed by creatine kinase. This enzyme transfers the phosphate group from phosphocreatine to adenosin5′-diphosphate (ADP) to generate cellular ATP. During ATP regeneration creatine kinase, by using ADP and H+, prevents muscle acidification and an increase in ADP concentration, which could inhibit ATP-dependent processes. The increase in adenosin5′-monophosphate (AMP) during long and exhausting work of muscles stimulates AMP-activated protein kinase (AMPK), which inactivates enzymes through their phosphorylation and stimulates AMP-activated protein kinase (AMPK), which deactivates enzymes through their phosphorylation and stimulates the oxidation of fatty acids as well as the transport and absorption of glucose in the muscles. A decrease in ATP levels may cause ADP to be converted to ATP and AMP via adenylate kinase, after which AMP is converted to inosin5′-monophosphate (IMP) by AMP deaminase with a concurrent release of ammonia (Grzyb and Skorkowski, 2008; Westerblad et al., 2010; Janicki and Buzala, 2013a). In horses, the concentration of IMP and ammonia in the muscles and blood increases during intense physical effort. As a result of metabolic processes, IMP can be further converted by inosine, hypoxanthine and xanthine to uric acid. The presence of these metabolites in blood indicates that ATP use in muscles has exceeded the ATP turnover rate and the adenine nucleotides were completely lost (Schuback and Essen-Gustavsson, 1998; Castejon et al., 2006).

The rate of CK release from the cells depends on its molecular weight and intracellular location, the type of attachment to different intracellular structures, the ratio of intracellular and extracellular concentration, as well as the extent and rate of muscle damage (Harris et al., 1998b). A physiologically greater amount of muscle CK is associated with the myofibrillar M-line, and a certain part of this isoform is loosely connected with band 1, as well as sarcolemma and endoplasmic reticulum (Rojo et al., 1991; Wallimann et al., 1992; Hornemann et al., 2003; Brancaccio et al., 2007). Creatine kinase is necessary for efficient function of the calcium pump by ensuring calcium ion homeostasis in extracellular water. The abnormal flow of Ca2+ ions through calcium channels in the cell, resulting from inhibition of the calcium pump in the endoplasmic reticulum, arrests Ca2+ ions in the sarcoplasm, which leads to a permanent muscle spasm (Grzyb and Skorkowski, 2008; Westerblad et al., 2010; Janicki and Buzala, 2013a).

Effect of oxidative stress on the activity of creatine kinase: Although horses are endowed with many different adaptive mechanisms for performing exercise tasks, these animal vulnerable to the consequences of excessive exercise loads. During maximal exercise in horses more than 8-fold increase of the heart rate was demonstrated, while in a highly trained men this value is increased only less than 4-fold (Butler et al., 1993; Art and Lekeux, 1995). In addition, there is also a more than twofold increase in the maximum oxygen consumption - VO2 max (ml/min/kg) and to the ratio of lung volume to body weight than in humans. Other examples of physiological adaptation of horses to intense physical effort is the ability to empty the spleen from red blood cells as a result of its autonomous contractions in the initial phase of exercise, maintaining hypoxia, and even excess CO2 in the blood during intense exercise (Bayly et al., 1989; Jones et al., 1989), and also the greater then in humans number of mitochondria in horse’s myocytes (Kayar et al., 1989).

During different forms of physical activity in horses, there is an increase in oxygen utilization by mitochondria in the active muscle cells, which generates more reactive oxygen species (ROS). Research results suggest that intense physical effort of horses may result in a high (even 60-fold) increase in oxygen consumption in energy processes, so these animals are particularly susceptible to excess ROS (oxidative stress) (Chiaradia et al., 1998). This phenomenon induces damage to cell lipid membranes as a result of intensive flow of electrons through the mitochondrial respiratory chain, ischemia and reperfusion, as well as high concentration of haem-containing proteins (Ji, 1999; Evans, 2000). The increase in cell membrane permeability is also associated with muscle hypoxia during intense aerobic effort (Harris, 1998b). Another, so to speak secondary source of damage initiated by mechanical injuries are peroxidation processes induced by intensification of oxygen-dependent activity of phagocytes (Jackson and O’Farrell, 1993; Aoi et al., 2004). These injuries, confirmed by histopathological examination, cause a release of many protein compounds (including CK) from the cytosol to the extracellular space (Valberg et al., 1993; Kinnunen et al., 2005b). The increase in ROS generation also results in changes in the antioxidant potential, which depend mainly on the duration and intensity of exercise, and on the degree of training, breed, sex, condition of horses, and the climatic conditions (Harris et al., 1998b; Piccione et al., 2007a, 2009; Lejeune et al., 2010; Soares et al., 2011; Janicki et al., 2013d).

Activity of creatine kinase during exercise of horses: The results of many observations made in horses, indicate that the duration and intensity of physical activity are major determinants of exercise-induced CK activity in the blood (Art et al., 1990; Stopyra, 2002; Jawor et al., 2007; Krumrych, 2007). Research carried out 3-month training under controlled conditions on a treadmill 5 times a week showed that CK activity before and after exercise fell within the physiologically normal range (Boffi et al., 2002). Standardized exercise test of horses on a treadmill submerged in water showed a significant increase in the plasma activity of this enzyme (Lindner et al., 2012). In studies, Anderson (1975) confirmed that the post-exercise
increase in CK activity is directly proportional to the duration of effort. This is confirmed by field observations, indicating that following a short-term effort increase in CK is smaller than in horses competing in endurance (Piccione et al., 2007a; Serteyn et al., 2009; Cywińska et al., 2012; Jagiří et al., 2012; Gondin et al., 2013).

Own research (Krumrych, 2007) also confirmed the relationship of the physical load value with changes in the activity of this enzyme. Post-exercise increase in CK was relatively low (5%) in recreational horses and significantly different from the results recorded in the standard exercise training event (41%), race (31%) and jumping (23%) horses. Relatively high CK values were also found in racehorses, which suggests that muscle microinjuries may occur as a result of even short but intense exercise. The results obtained are correlated to the observations of other authors on horses subjected to different exercise loads (Art et al., 1990; Stopyra, 2002; Jawor et al., 2007), with the highest post-exercise increase in CK activity observed after long-distance rides (Kerr and Snow, 1991; Hargreaves et al., 2002) – immediately after a 140-km ride, the activity of this enzyme increases as much as 10-fold (Marlin et al., 2002). According to some authors, the increase in muscle enzyme activity in the blood plasma is common in endurance horses and should not be interpreted as a side effect, unless it is accompanied by clinical signs of myopathy, and the increased activity of this enzyme in the blood plasma lasting a long time (Nimmo and Snow, 1982). The study of Szarska (2001) and Cywińska et al. (2012) showed that the extent of change in the enzyme activity depends on the length of distance covered. The plasma activity of creatine kinase in horses after endurance exercise on the 120 km increased about 14-fold compared with before the exercise (Serteyn et al., 2009). Another study (Cywińska et al., 2012) showed that CK activity increased almost 18-fold after prolonged exercise. It should be noted that exercise-induced increase in CK activity is temporary. In horses, the activity of this enzyme may easily double without noticeable clinical consequences, and the values of 2,000-3,000 U/L post exercise are often observed in long-distance horses that complete the race. It seems that this phenomenon may reflect the adaptation of skeletal muscles to the exercise loads (Cywińska et al., 2012) as a result of strengthening the muscle cell membranes and connective tissue, as well as conversion of type IIx fibres to tougher type IIB fibres and a decrease in oxidative stress by increasing the activity of antioxidant enzymes (Lindner et al., 2006).

The results of many studies suggest that increased activity of CK in the blood occurs during intensive exercise (Milne et al., 1976; Pösö et al., 1983; Harris et al., 1990), while smaller loads do not cause a significant increase in the activity of this enzyme (Table 1). No changes in CK activity were found in foals as a result of standardized daily training during 140 days (Tateo et al., 2008). In horses, prior to 14 days standardized exercise test, activity of CK in blood plasma was increased only slightly (and non-significantly) immediately after exercise (Escribano et al., 2011). Slightly higher values of this enzyme were reported by Kędzierski (2009) for horses aged 2-4 years during standard exercise, done 5 days per week for one month. Another study found CK activity to gradually and significantly increase until 60 days of training and to decrease in the final stage of training (80 days) during standard training of horses aged 3-4 years (Fazio et al., 2011). In a study by Janicki et al. (2013b) recorded a trend to a small, progressive increase in resting activity during the 100-day performance test. Because the exercise load did not change towards the end of training, the muscle cells have probably adapted to the load applied (Lindner et al., 2006). When the cyclic submaximal loads ended, CK activity in the plasma of horses was significantly higher than after a short test of

<table>
<thead>
<tr>
<th>Table 1: Mean activity of CK in adult horses in physiological conditions depending on different types of exercise</th>
<th>CK (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic of exercise</td>
<td>At rest</td>
</tr>
<tr>
<td>Short, intense exercise on a treadmill</td>
<td>147</td>
</tr>
<tr>
<td>Recreation exercise</td>
<td>219</td>
</tr>
<tr>
<td>Police horses on duty</td>
<td>165</td>
</tr>
<tr>
<td>Polo competition</td>
<td>144</td>
</tr>
<tr>
<td>Jumping training</td>
<td>164</td>
</tr>
<tr>
<td>Jumping training</td>
<td>192</td>
</tr>
<tr>
<td>Jumping competition</td>
<td>120</td>
</tr>
<tr>
<td>Jumping competition</td>
<td>179</td>
</tr>
<tr>
<td>Race training (1000±200 m)</td>
<td>95</td>
</tr>
<tr>
<td>Race competition (1800 m)</td>
<td>136</td>
</tr>
<tr>
<td>Race treining</td>
<td>221</td>
</tr>
<tr>
<td>Trotters treining</td>
<td>238</td>
</tr>
<tr>
<td>Driving treining</td>
<td>207</td>
</tr>
<tr>
<td>Endurance training (30 km)</td>
<td>176</td>
</tr>
<tr>
<td>Endurance rides (34-60 km)</td>
<td>180</td>
</tr>
<tr>
<td>Endurance rides (40, 80, 120 km)</td>
<td>246</td>
</tr>
<tr>
<td>Endurance rides (70, 100 km)</td>
<td>245</td>
</tr>
<tr>
<td>Endurance rides (80 km)</td>
<td>277</td>
</tr>
<tr>
<td>Endurance rides (80 km)</td>
<td>275</td>
</tr>
<tr>
<td>Endurance rides (91 km)</td>
<td>131</td>
</tr>
<tr>
<td>Endurance rides (120 km)</td>
<td>191</td>
</tr>
<tr>
<td>Endurance rides (121 km)</td>
<td>222</td>
</tr>
<tr>
<td>Endurance rides (140 km)</td>
<td>222</td>
</tr>
<tr>
<td>Endurance rides (120-160 km)</td>
<td>167</td>
</tr>
<tr>
<td>Endurance rides (120, 160 km)</td>
<td>382</td>
</tr>
<tr>
<td>Endurance rides (164 km)</td>
<td>318</td>
</tr>
<tr>
<td>Endurance rides (166 km)</td>
<td>155</td>
</tr>
</tbody>
</table>
high intensity. The period of preparation for increasing exercise loads has a significant effect on CK activity in the blood while reducing the horse’s response to effort (Siciliano et al., 1995). The differences in post-exercise CK activity reflect different exercise load of horses, depending on the type of use. Higher mean activity of this enzyme was observed after exercise in event and racing horses. More than 60% of the animals of these groups demonstrated significantly higher after exercise (Krumrych, 2007), but in the range of physiological standards (Muñoz et al., 2012) CK activity (60-340 IU/L), compared with the other horses. This result implies a relationship of intensity of damage muscle cells, as in the whole course of the training session event horses registered the highest mean value of %VO2max while the racehorses were characterized by far the largest mean value of this ratio in the phase of intensive effort.

On the resting CK activity in horses affected, except to the intensity and duration of exercise, many other physiological factors such as age, breed or circadian rhythm. In foals, aged between 3 and 6 months was observed the largest resting activity of CK in blood plasma (Fig. 1). It is considered to be associated with increased muscle activity during the weaning period, although foals generally have higher CK levels than adult horses (Muñoz et al., 2012). In a study by Janicki et al. (2013b), mean resting activity of CK in the serum of 3- to 4-year-old stallions during a 100-day performance test fell within the physiologically normal range for the species. Jawor et al. (2007) showed the mean resting levels of CK activity in the blood of endurance horses to be significantly higher than in jumping, race and event horses. It was also found that in the developing countries, cold-blooded horses working in adverse conditions are characterized by higher CK activity in blood compared to warm-blooded horses in the developed countries (Pritchard et al., 2009). A reliable assessment of CK activity should also account for the circadian rhythm. The acrophase (time of peak) during daily rhythm of CK activity was observed in horses during the photophase (period of light) before exercise (11:02-10:26 AM) and post exercise (2:51-3:56 PM), and during the scotophase (the dark phase) (7:08-6:25 PM) immediately after exercise (Piccione et al., 2009).

Diagnostic usefulness of determining creatine kinase during physical effort of horses: After intense exercise, the blood concentration of CK peaks within 4 to 6 hours and its half-life is 90-120 minutes, which makes CK a reliable and relatively sensitive indicator for detection and monitoring of muscle damage in the horse (Siciliano et al., 1995; Harris et al., 1998b; Boffi et al., 2002; Chaney et al., 2004). This enzyme is thus relatively quickly removed from the blood, and the high CK values return to normal in a short period of time (Harris et al., 1998b; Aoki and Ishii, 2012). Interpretation of the results is complicated by large variation in the activity of this enzyme as a result of different exercise intensity and duration regimens used, together with varying sampling intervals and the possibility that the studies may have included horses with possible muscular problems (Muñoz et al., 2002; 2010; 2013). It is suggested that the sampling at least 24 h after exercise can be helpful in the diagnosis of skeletal muscle damage. This allows to distinguish horses showing normal physiological response to exercise and those with abnormal or pathologic response to the applied load (Harris et al., 1990). The blood is most commonly collected directly before and after (up to 5 min.) exercise and 15, 30, 60 minutes or 24 hours after the exercise (Kanter et al., 1988; Räisänen, 1995; Castejon et al., 2006; Krumrych, 2007; Jawor et al., 2007; Piccione et al., 2007a, 2010; Escribano et al., 2011; Soares et al., 2011; Zobba et al., 2011; Janicki et al., 2013b). It should be noted, however, that a delay in analyzing CK activity in the blood collected from horses may cause an additional increase in CK induced by adenylate kinase being released from erythrocytes and free hemoglobin. This enzyme may also disturb the assessment of the activity of other analytes and adversely affect the diagnostic interpretation of the results obtained (Rendle et al., 2009).

The measurement of CK activity before and after exercise is helpful in diagnosing some pathological conditions of the muscles and can be a reliable indicator of overtraining in horses (Padalino et al., 2007; Aoki and Ishii, 2012). According to many authors, plasma activity of CK in clinically healthy horses ranges between 90 and 275 U/L (Siciliano et al., 1995; Muñoz et al., 2002), although Ostaszewski et al. (2012) believe that the maximum value should not exceed 200 U/L. As a result of intense exercise, serum activity of CK may increase as much as 35-fold (Siciliano et al., 1995). It should be noted at the same time that this reaction is often unrelated to any external clinical signs of myopathy in horses. Post-exercise increase in the activity of CK in blood is mainly due to a change in cell membrane permeability rather than muscle cell necrosis. It was found that only very high levels of CK activity in blood plasma (>10,000 U/L) reflect a considerable disintegration of skeletal muscles in horses (Siciliano et al., 1995). One such disease is recurrent exertional rhabdomyolysis a hereditary disorder of calcium metabolism with concurrent changes in gait

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**Table 2: Mean activity of CK in adult horses with exertional rhabdomyolysis**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>CK (IU/L)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected</td>
<td>&gt; 1,500</td>
<td>McEwen and Hulland (1986)</td>
</tr>
<tr>
<td>Day 1 after competition</td>
<td>10,340</td>
<td>Muñoz et al. (2013)</td>
</tr>
<tr>
<td>1.5 hrs after stopping exercise</td>
<td>12,430</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>56,103</td>
<td>El-Ashker (2011)</td>
</tr>
<tr>
<td>Severe</td>
<td>267,906</td>
<td></td>
</tr>
<tr>
<td>Survivors</td>
<td>73,999</td>
<td></td>
</tr>
<tr>
<td>Non-survivors</td>
<td>241,164</td>
<td>El-Ashker (2012)</td>
</tr>
</tbody>
</table>

**Fig. 1:** The range of values CK (IU/L) in resting foals depending on month of age (Muñoz et al., 2012).
that horses (Evans, 2007). Measurement of CK activity after maximal exercise may be also useful for diagnosing polysaccharide storage myopathy (PSSM). This disease, which is mainly found in Quarter Horses, is related to abnormal glycogen metabolism in skeletal muscles. CK activity in the serum of these horses increases considerably 4 h after maximal and submaximal exercise and is significantly higher in affected compared to healthy horses of this breed. However, submaximal exercise is more commonly used in sport and recreation to healthy horses of this breed. Therefore, the measurement of CK activity after physical exercise may be a more effective tool in the monitoring of this disease in horses (Evans, 2007).

It has recently been shown (Ostaszewski et al., 2012) that β-hydroxy-β-methylbutyrate (HMB) may strengthen the muscle cell membrane during intensive exercise in horses. Giving horses 15 g CaHMB/day caused a significant decrease in exertion-induced increase of CK activity in blood plasma. Another effective antioxidant that reduces the release of CK during exercise in horses is γ-oryzanol, which, as a potent inhibitor of reactive oxygen species generation, protects lipid cell membranes from peroxidation, thus reducing their permeability.

**Conclusion:** Determine the activity of creatine kinase may be a reliable indicator of the impact assessment exercise in horses. The CK activity during exercise depends largely on the duration and intensity of exercise. These factors may affect different CK activity during exercise in horses as well as age, breed or circadian rhythm. According to many authors, plasma activity of CK in clinically healthy horses ranges between 90 and 275 U/L. However, the increase above this range, the activity of CK in the blood plasma is a frequent phenomenon in endurance horses and should not be interpreted as an undesirable symptom, unless it is accompanied by clinical signs of myopathy or persisting over a longer period of time. Also the activity of this enzyme in the blood. Dispersion of the results on CK activity shown in the literature may also result from different time of blood sampling from horses after exercise. It is recommended to collect blood from the horse directly before and after (up to 5 min.) exercise and 15, 30, 60 minutes or 24 hours after the exercise. Due to the various analytical methods applied and various units in which the results are presented, it seems that it would be appropriate to present variability of post-exercise CK activity in percentages, which will facilitate the assessment of this phenomenon in different individuals, often subjected to very different loads. In addition, further research on CK in horses should include determining the activity of the muscle isoform of creatine kinase (M-CK), which is only released from skeletal muscle cells. This will allow a precise assessment of the impact of the applied loads on the status of skeletal muscle cells than total CK activity in the organism of horses.

**REFERENCES**


Kayar SR, H Hoppeler, SL Lindstedt, HU Lindstedt, JS Hoppeler, HU Claassen, JH Jones, B Essen-


