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PHYSIOLOGICAL RESPONSES AND MOLECULAR SIGNATURES OF EXERCISE IN HORSES

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Abstract

Exercising equines, especially horses, exhibit significant changes in the physiological responses and several other vital signs. The prominent observable physiological indices are the heart rate (HR), velocity at peak Heart rate (V_{200}), and lactate $V_{[La]}$. The blood lactate is the biochemical signature of muscular fatigue. Trainings and conditioning schedules have been devised to include the speed at blood lactate levels of 2.0 and 4.0 mM/l. These indices along with recovery of heart rate and blood lactate are important indices for judging the performance of exercising horses. Adaptation to exercise with proper training results in development of cellular tolerance in the exercising muscles and other tissues. With the development of next generation technologies, the analysis of blood and muscle transcriptomes in exercising horses has led to identification of performance genes in race horses. Myostatin (MSTN) genotyping is used to identify and select the horses at early stages for either fast sprint or long distance endurance events. Genes of immune deregulation, mitochondrial respiration, oxidative phosphorylation, tissue repair, tissue remodelling and specific cytokines are among the *up regulated genes in the race horses while the most suppressed genes are the ones involved in signal transduction, cell cycle regulation and protein synthesis. Further research is required in the future, to identify the molecular signatures of stress tolerance in horse breeds other than thoroughbreds and race horses, which have evolved and adapted to the climate and utility at specific locations in the world. Such an information would facilitate the horse breeders to devise selection plans in order to improve the exercise and work performance. This would also facilitate diagnosis of the diseases and metabolic disorders that are caused by exercise induced stress.*

Key words*: horses, molecular signatures, exercise, genes, performance.*

INTRODUCTION

Horses (*Equus ferus caballus*), belong to equines, which are taxonomically the members of genus *Equus* under the family family *Equidae*. Equines include modern horses, zebras, asses and hybrids obtained from them. Domesticated equines (horses donkeys, mules) are reared in all weather types (ambient temperature ranging from -40ºC to higher than 40ºC (Cymbaluk and Christison , 1990). Horses are best among the domesticated animals in terms of adaptation and performance for intense exercise. This is due to the special modifications in the structural and physiological system in comparison to the other livestock species.

Identification of perfect diagnostic markers of performance equines has long been the interest of breeders and race horse owners. Since ages, selective breeding has been followed in the various horse breeds all over the world. With the advent and ever continuing refinement of high throughput sequencing techniques, the complete horse genome has been sequenced for different breeds starting with a thoroughbred mare, 'Twilight' in 2009 (Wade et al., 2009, Jun et al., 2014). The genome sequences are now made available publicly for annotations and related analyses. This has enormously facilitated the characterization of various tissue specific transcriptomes and genome wide polymorphisms leading to the identification of candidate genes and establishment of definitive markers of performance equines in the last 5 years.

This paper presents an overview of the physiological and molecular signatures in response to exercise and exercise induced stress in horses. The knowledge of molecular mechanisms of stress response in equine athletes can allow us plan an appropriate and high-grade training programs to obtain better performance and to preserve horse welfare (Capelli et al., 2007, Capomaccio et al., 2013).

PHYSIOLOGICAL RESPONSES TO EXERCISE IN HORSES.

Types of exercise in horses:

Exercise is manifested in the horses by three kinds of movements: trot, canter and gallop.
The flat races are conducted by International Federation of Horseracing Authorities (IFHA), while Fédération Equestre Internationale (FEI) conducts endurance, jumping, dressage and para, eventing, vaulting and reining. The flat races comprise of sprint : 5-6 furlongs, ≤ 1300m; Mile: 6.5-9.49 furlong, 1301-1900m; Intermediate, 9.5-10.5 furlong, 1901-2112m; Long : 10.51-13.5 f, 2114-2716m (Hill et al., 2012; www.horseracingintfed.com).
Steeplechase or, National Hunt racing (in UK and Ireland) involves horses running over obstacles, fences over distances upto 4.5 miles (7200m). Endurance races are long distance cross country runs (40 -160km) and may be run over two days. All these races involve extreme physical and psychological activity and therefore, the code of conduct of these organizations implies that the horse welfare must take precedence over all other demands and horses must be fit, competent and in good health before they are allowed to compete (http://fei.org/fei/about-fei/values, Munsters et al., 2014).
Intensity wise, the light, medium and heavy work or exercise/training is classified as per Nutrient Requirement for Horses (NRC, 2007) as given in Table 1. Exercise physiologists devise their own standardized exercise tests (for details refer to reviews by Courouce, 1999; Munsters et al., 2014) on treadmill or field so as to test the performance of the horses.
The tests generally comprise of initial warm up followed with incremental steps at increasing speeds with or without angle of tilt on the treadmill or loads when working with draught horses. The time duration of incremental steps are designed to mimic the conditions in the flat racings. Such a time period allows for development of lactate threshold in the animal.

Table 1. Exercise classification as per Nutrient Requirements for Horses, NRC (2007)

Exercise Category	Mean Heart Rate	Description	Types of Events
Light	80 beats/ minute	1-3 hours/week 40% walk, 50% trot, 10% canter	Recreational Riding Beginning of Training Program Show Horses (occasional)
Moderate	90 beats/ minute	3-5 hours/week 30% walk, 55% trot, 10% canter 5% gallop, jumping, other skill work	Recreational Riding School Horses Beginning of training/breaking Show Horses (frequent) Polo Ranch Work
Heavy	110 beats/ minute	4-5 hours/week 20% walk, 50% trot, 15% canter 15% gallop, jumping, other skill work	Ranch Work Polo Show Horses (frequent, strenuous events) Low/medium level eventing Race Training (middle stages)
Very Heavy	110-150 beats/ minute	Varies; ranges from 1 hour/week speed work to 6-12 hours/week slow work	Racing Elite 3-day event

Physiological responses, lactate and fitness indices:

The prominent physiological responses of exercise in equines are vital signs, i.e., pulse rate, respiration rate, rectal temperature, skin temperature, dehydration, capillary refill time, jugular refill time, muscular tone, gut sounds, anal tone, gait, cardiac output, the maximal oxygen uptake (VO_2) and blood lactate. Treadmill and field tests have relied on these responses to evaluate the fitness and performance of exercising horses. Equines, in particular horses, have several well developed anatomic features in comparison to the other domesticated livestock species which provide

them with improved athletic performance. For instance, anatomically their heart has a high cardiac capacity that can increase by a factor of 10 than at rest, and ventilation of lungs by 30 (Art and Lekeux, 2005). Thus their maximal maximal oxygen consumption VO_2 can also increase by a factor of 60 (Eaton, 1994; Art and Lekeux, 2005). Fitness indices for the exercising horses are calculated from the relationship between heart rate, blood lactate and velocity at particular heart rate and blood lactate (La) during exercise and in the recovery period. The velocity leading to buildup of a particular blood lactate $V_{[La],}$ (V_4) at blood lactate concentration of 4 mM/l is used to determine fitness of horses (Linder et al., 2009; Munsters et al., 2014). It is referred to as 'lactate threshold', the point of onset of lactate accumulation and thereby aerobic capacity of the horse (Courouce, 1999). The curve of blood lactate plotted against speed would help extrapolate the aerobic threshold of the horses and is defined as the level of work just below that at which metabolic acidosis occurs (Wasserman et al., 1973; Munsters et al., 2014). Alternatively in conjunction with the V_4, V_{200} is also used to train the horses. V_{200} is the velocity at which heart rate of 200 beats per minute is reached and is considered as an useful index for comparison of the cardiovascular capacity of the horses (Persson, 1983; Courouce, 1999; Ohmura et al., 2013). Recovery at 5 and 10 min post exercise or after the last step of the standardized exercise test (HR_{rec5} and HR_{rec10}) is also recorded for determining the fitness (Munsters et al., 2014; Kumar et al., 2015). In most of the mature horses, the workload carried out at V_{200} is close to V_4 (658 and 656 m/min respectively in French trotters, Courouce 1999). Both V_4 and V_{200} increases with training in the horses (von Wittke et al., 1994; Eaton et al., 1999; Lindner et. al., 2009). On 2 year old Haflinger stallions, Lindner et al. (2009) showed that the largest mean increase of 7.3% in V_4 after conditioning when horses were exercised during 45 min at their $V_{1.5}$ as compared with 4.9 and 2.3% increase for exercise at $V_{2.5}$ for 45 min and V_4 for 25 min, respectively. For V_{200}, the largest increase was calculated in the horses exercising at their $V_{1.5}$ during 45 min (9.6%), followed by exercise at V_4 during 25 min (6.9%) and at $V_{2.5}$ during 45 min (6.4%). Mean speed of horses during exercise was 3.1, 3.4, and 3.8 m/s for $V_{1.5}$, $V_{2.5}$, and V_4, respectively. The lactate [La] after exercise ranged from 1.60 to 6.70 mM/l after 45 min of exercise at $V_{1.5}$, 1.35 to 6.85 mM/l after exercise $V_{2.5}$, and 2.15 to 8.45 mM/l after 25 min of exercise at V_4. These observations confirm that conditioning schedules in the routine exercise training programmes enhance oxygen carrying capacity and lactate tolerance in the exercising muscles and other tissues.

The heart rates of horses during intense maximal exercise can increase from 30-40 at rest to as high as 240 beats per minute (bpm), (Ohmura et al., 2013, Munsters et al., 2014) and can return back to 64 bpm within 20 minutes post exercise in well trained horses. The mean heart rates of 2-yr-old Haflinger stallions (n=6) during exercise ranged between 148-176; 153-185; and 165-195 beats/min after running to velocity, $V_{[La]}$ of $V_{1.5}$, $V_{2.5}$, and V_4, respectively (Lindner et al., 2009).

In young Friesian horses on the standardized exercise test, the heart rates at walk (2m/s), trot(3.5m/s) and canter (5m/s) were 75-99, 128-148, and, 159-190 beats/min respectively while the mean heart rate at rest was 41±8 beats/min (Munsters et al., 2013). In the same study, heart rate recovered to 90±9 beats/min at 10 min after a peak of 192±13 beats/min during the standardized test. In Marwari mares, run to a distance of 10 km in sandy terrain at a mean speed of about 5m/s, Kumar et al. (2015) reported the pulse rates of 41±1.00, 72.25±5.92, 63±3.0, and 51±5.20 pulse per min for pre-trail, and at 5, 10 and 20 min post trail ride respectively. The corresponding values for respiration rates were: 8±0.71, 45.75±14.17, 42±13.13 and 18±4.32 respirations per minute (rpm), for rectal temperature, 99±0.22, 102.83±0.28, 102.43±0.16 and 102.05±0.17ºF, for skin temperature, 99±0.22, 102.83±0.28, 102.43±0.16 and 102.05±0.17ºF respectively.

All other vital signs were found to return to safe limits at 20 min post exercise. The faster the recovery better is the horse in terms of adaptation and fitness. For endurance races, FEI norms for pulse rate are 64 bpm to recover within 30 minutes of an endurance races of atleast 20 km in order to considered fit to undergo the further distance. Quicker the

return, better the score accorded to the horses. The horses are also required to be fit 24 h after the race. The lactate has been observed to rise to as high as 38.5 mM/l in untrained horses during instant high intensity exercise . High lactate production above the threshold causes leads to its accumulation in the cells and the lactate may thus get released in to the blood stream. This causes fatigue in the cells that have resorted to anaerobic respiration and a reduction in the performance. In trained equine athletes, the increase in plasma or blood lactate is transient and would return back to near normal values in 30 minutes of rest post exercise. This has been shown in three studies in polo ponies. The lactate levels increase significantly after the match (chukkas) to 10.24 mM/l from 1.21 mM/l and returned back to 4.7 mM/l after 30 minutes of recovery period (Zobba et al., 2011). The increase after the match in other studies were 9.2 mM/l (Craig et al., 1985) and 18.70 mM/l (Ferraz et al., 2010). Ohmura et al., (2013) also reported a very high increase in the lactate level (12.6 to 18.3 mM/l) during the once per week high intensity treadmill exercise in thoroughbred horses.

In horses submitted to a 500m full gallop, mean lactate time to peak (LP) was 8.2±0.7 mM/l at approximately 5.8±6.09 min, mean Lactate Minimal Speed (LMS) was 20.75±2.06 km/h and mean heart rate at LMS was 124.8±4.7 BPM. Blood lactate remained at rest baseline levels during 10,000 m trial at LMS, but reached a six fold significantly raise during 10% above LMS trial after 4000 and 6000 m ($p<0.05$) and ($p<0.01$) after 8000 and 10,000 m (Gondim et al., 2007) In untrained thoroughbreds, exercised in six incremental steps to maximal heart rate or fatigue with mean maximal velocity of 12.4m/s, mean distance of 4362 m and mean time of 8.77 min, the maximal heart rate reached 218 beat per min, and mean post exercise lactate was 13.3 mM/l. (Mc Givney et al., 2009).

Field and standardized treadmill tests could discriminate the endurance horses based on $V[la_4]$ and V_{200} in which trained participants of 120 km or more race had these indices higher than the horses of lower race levels (Fraipoint et al., 2012).

MOLECULAR SIGNATURES OF EXERCISING HORSES.

Each of the physiological and biochemical indices and signatures of the exercising equine is controlled at the molecular level. The genes controlling these behavioral and cellular adaptations are the control centres of the performance in equines. Although the horse genome was sequenced after the genomes of many other livestock were sequenced, there is wide utility of the horse genome in understanding the molecular mechanisms of human physiological process and disorders as there is a strong conserved synteny of the chromosomal arrangement of genes in humans and horses (Wade et al., 2009; Schröder et al., 2011). Evolutionary analysis through analysis of differentially expressed genes pre and post exercise in an RNA-seq revealed that, during the period of horse domestication, the older layer is mainly responsible for adaptations to inflammation and energy metabolism, and the most recent layer for neurological system process, cell adhesion, and proteolysis (Kim et al., 2013).

The instant and long term changes in the physiological functions as a result of exercise in the horses are brought about at the molecular level by transient changes in the gene transcriptions. Early studies to identify exercise induced transcripts revealed the up regulation of interleukin-8 (IL-8) and Matrix metalloproteinase-1 as the gene transcripts upregulated in the peripheral blood mononuclear cells immediately post exercise in the high level performer Arabian horses in 90-160km endurance race and levels returned to basal levels after 24h of the competition (Cappelli et al., 2007, 2009). The down regulated transcript fragments were similar to that of human HSP90, retinoblastoma binding protein 6(RBBP6) and eukaryotic initiation translation factor gamma 4 (IF4G3). The studies showed that endurance race induces inflammatory response in the blood cells which inturn stimulates production of anti-inflammatory chemokines (IL-8) and tissue remodelers such as MMP-1. The reduced expression of HSP90 and RBBP6 indicated the reduction of lymphoproliferative response as a cause of stress related immune depression

(Cappelli et al., 2007). In another study based on qRT-PCR of skeletal muscle mRNA, the effect of adaptation of the energy homeostasis and cellular respiratory mechanisms in trained horses (Eivers et al., 2010) were evident. While the transcripts involved in energy homeostasis in the muscle (CKM), mitochondrial respiration and oxidative phosphorylation (COX4I1), glucose transport (SCL2A4), mitochondrial biogenesis (PGC-1α) and PDK4 were upregulated in the untrained horses after 4h of exercise, no such changes were observed either immediately after exercise or at 4h after exercise. Ten months of training, resulted in a subsided the up regulations of most of the genes except for PDK4 and PPARGC1A, and COX4I2, was down regulated. CKM is the creatine kinase muscle specific gene and encodes creatine kinase enzyme muscle isoform. The enzyme catalyses the conversion of creatine phosphate and ADP to creatine. During excessive stress that leads to altered membrane permeability, the creatine kinase often leaks into blood. High serum levels of the enzyme are detected at 4-6 h post exercise (MacLeay et al., 2000). In comparison to 1% of human skeletal muscle transcriptome, equine CKM transcript represents about 6.9 % of the total annotated equine transcriptome signifying the highly adapted athletic capacity of the equine muscles in the thoroughbreds (McGivney et al., 2010). The G allele, in the polymorphism for CKM (CKM g.15884567A>G) has a role in disrupting the binding site (GCA/GA) of interferon regulatory factor-1 (IRF-1) while the A allele retains the site (GCAA). In humans, IRF-1 is shown to be significantly activated after endurance exercise (Mahoney et al., 2005). The A allele was found to be in favour of the elite performance (Gu et al., 2010).

COX4I1 and COX4I2 are the cytochrome c oxidase genes identified in the oxidative phosphorylation pathway in KEGG pathway. The COX4 enzymes transfer an electron from the reduced cytochrome c to oxygen during mitochondrial respiration (Gu et al., 2010). COX4I1 is preferentially transcribed in normoxic environment while the reverse is true for COX4I2. The increased basal COX4I1 activity was observed with training and post exercise was positively related with the athletic ability of thoroughbred horses reflecting a long term adaptive response so as to increase mitochondrial respiratory capacity (Eivers et al., 2010). An intronic SNP in the COX412 gene g.22684390C>T has been reported to be associated with the racing performance of Throughbred horses (Gu et al., 2010). The favourable allele (T) retains the glucocorticoid response element (GRE) binding site (TGTT) while the less favourable allele (c) disrupts the site (CGTT), thereby disabling the glucocorticoid response element (GRE) binding and repressing gene expression.

SCL2A4 (Solute carrier family 2 (facilitated glucose transporter), member 4) regulates the glucose uptake by the exercising muscles. PPAR GC1A (also known as PGC-1α) is a key regulator of the mitochondrial biogenesis (Wright et al., 2007) and may influence lactate uptake into skeletal through monocarboxylate transporter, MCT1 (Benton et al., 2008; Eivers et al., 2010, 2012).

In the subsequent years, the majority of research focused on high throughput sequencing technologies such as Microarray technology, digital gene expression (Gu et al., 2009; McGivney et al., 2010; Capomaccio et al., 2010), whole genome sequencing and RNA-sequencing to identify the molecular signatures **(** Park et al., 2012; Kim et al., 2013; Capomaccio et al., 2013**).** The real time qRT-PCR was still the prime method to confirm the regulation of the most abundant, up regulated and down regulated transcripts identified by high throughput techniques.

Global mRNA expression profiling of the exercising horse muscle and peripheral blood mononuclear cells got initiated from 2009 when McGivney and coworkers reported the first global transcriptome of Thoroughbred skeletal muscle during exercise. In untrained horses, exercised in six incremental steps to maximal heart rate or fatigue, seven probes that were highly upregulated (> +1.5 fold), belonged to the FOS (v-fos FBJ murine osteocarcinoma viral homolog gene), HSPA1A (heat shock 70kDa protein 1A gene), PFKFB3 (6-phosphofructo-2-kinase/ fructose-2,6 biphosphatase 3 gene), EGR1 (Early growth response 1 gene). HSPA1A, FOS and EGR1 are members of the immediate-early response gene family (McGivney et al., 2009). While the

expression of FOS, EGR1 and PFKB3 returned back to basal levels at 4h post exercise, the HSPA1A expression continued to remain higher. The PFKFB3 gene encodes the product which is involved in energy sensing and metabolism. Exercise stimulated glucose deprivation and hypoxia, are the prime factors responsible for its increase immediately after exercise. The prominent among the signifcantly down regulated genes (>1.5 fold) were CWF19L2 (CWF19-like protein gene), UXS1 (UDP-glucuronic acid decarboxylase 1 gene),TXNL5 (Thioredoxin-domain containing protein 17 gene), PCOLCE2 (Procollagen C endopeptidase enhancer 2 precursor gene), TRAM 1 (translocation –associated membrane protein 1 gene) and ROBO (roundabout homolog 1 precursor gene) (McGivney et al., 2009). The heat shock family proteins are the molecular chaperones associated with the transport of various nuclear encoded proteins in to the mitochondria and translocases of the outer membrane complex proteins to the mitochondrial surface in contractile muscles, thereby providing protection against cellular damage that could be caused by reactive oxygen species (McGivney et al., 2009).

Differentially regulated functional group categories and genes

Most abundant mRNA transcripts in the equine muscle transcriptome were the ones involved in muscle contraction, aerobic respiration and mitochondrial function (McGivney et al., 2010). All studies involving high throughput sequencing technologies have reported functional analysis of the expressed genes using various bioinformatics softwares on gene ontology groups and KEGG pathways. Functional groups to which the up regulated mRNA transcripts (post training as compared to pre training levels) were assigned included contractile fibre, muscle contraction, metabolic process, electron carrier activity, ribosome, translation regulator activity TCA cycle, mitochondrion, oxidative phosphorylation, fatty acid metabolism (McGivney et al., 2010; Park et al., 2012); and inflammatory response, haematological system development and function, haematopioseis, immune cell trafficking; and antigen presentation in the ingenuity pathways (Capomaccio et al., 2010, 2013, Kim et al., 2013). IL1R2 (Interleukin 1 receptor Type II), MMP1 (Matric metallopeptidase 1 (interstitial collagenase), IL-18 (Interleukin-18), STON2 (Stonin 2), CEBPB (CCAT/enhancer binding protein (C/EBP) beta, CXCL2 (Chemokine (C-X-C motif)ligand 2, FST (Follistatin), IL-8 (interleukin 8) were significantly up regulated genes in these pathways; while, LCK (Lymphocyte-specific protein-tyrosine kinase 3), FCER1A (FFc fragment of IgE. high affinity I. receptor for; alpha polypeptide), MAP3K1 (Mitogen-activated protein kinase 1), STAT4 (Signal transducer and activator of transcription 4), CCL5 (Chemokine (C-C motif) ligand 5), ELK4 (ELK4. ETS-domain protein (SRF accessory protein 1), COX7A2L (Cytochrome c oxidase subunit VIIa polypeptide 2 like) genes were the significantly downregualted gene transcripts (Capomaacio et al., 2010). Through Microarray analysis, the gene ontology functional groups with significantly decreased expression post training compared to pre training levels included phosphate transport, inorganic ion transport, positive regulation of epithelial cell function, cytoskeleton, sarcoplasm (McGivney et al., 2010). KEGG pathway categories up regulated (post training as compared to pre training levels) were complement and coagulation cascades, vitamin B6 metabolism, folate biosynthesis, tyrosine metabolism, nicotinate and nicotinamide metabolism; and the significantly down regulated as Tight junction, JAK-STAT Pathway, Long term potentiation, Cell communication, ECM-receptor interaction and the ones with decreased expression (McGivney et al., 2010).

By RNA sequencing the exercise transcriptome in high level performer horses in the endurance races, CXCR4, integrins (ITGAL and ITGAM), kinases (MAP3K4 and MAPK14) and metalloproteinases (MMP1, -8, -25, and -27) were observed to be upregulated (Capomaccio et al., 2013). The most highly upregulated gene in their study was IL22A2 (interleukin-22 A2), which encodes IL22 binding protein (IL22BP; a soluble receptor of IL22). This gene is implicated in several chronic inflammatory diseases (Beyeen et al., 2010). The most highly down regulated genes were related to protein

synthesis, growth factors, signal transducers, and cell cycle regulators (GATA2, BMP2, GPR56, FLT4), all being well established as suppressed during severe stress (De Nadal et al., 2011). Up-regulated genes in both muscle and blood tissue is in response to the exercise induced inflammation and involves removal of necrotic tissue, repair of injured muscle, nerve fibres, blood vessels and extracellular matrix (Kim et al., 2013).

MSTN gene sequence polymorphism:

Equine Myostatin gene is located on the chromosome ECA18 and encodes skeletal muscle specific protein Myostatin (Caetano et al., 1999; Hosoyama et al. 2002). The Myostatin negatively regulates the skeletal muscle fibre growth and proliferation, thereby limiting the skeletal muscle mass (McPherron et al., 1997; Hill et al., 2011; Dalll'Olio et al., 2010; Tozaki et al., 2011; Hill et al., 2012a; 2012b). Of as many as six sequence polymorphisms in the equine Myostatin (MSTN) gene, the most important and widely applied polymorphism fin horse race industry is the g.66493737C/T, This polymorphic gene is associated with the best race distance among elite racehorses (Hill et al., 2010, 2012; Dalll'Olio et al., 2010; Tozaki et al., 2011). The C/C horses are suited to fast, short-distance races; C/T horses compete favorably in during middle-distance races; and T/T horses have greater stamina (Hill et al., 2010, 2011). Hill et al (2011) further provided the speed indices related with theses alleles in the horses. In thoroughbreds, the C allele g.66493737C/T is found in very high frequency and therefore confirms selective breeding of this horse for short fast sprint races for more than 300 years down the history. C/C and C/T thoroughbred horses outperformed all other genetoypes, thereby providing the most important selection criteria for the horse race industry i.e., atleast one C is required to improve speed (Hill et al., 2012). This SNP is found in the intron 1 region of the gene and the optimum distances was found to be 1000-1600m (50-8f) for the C/C thoroughbred horses, 1400-2400m (7-12f) for C/T horses, and >2000m for T/T horses (Hill et al., 2010). The gene is now dubbed as 'The Speed Gene' and exclusive licence for commercial use has been granted to the biotechnology company, Equinome Ltd. (Hill et al., 2012) thereby establishing the commercial application of MSTN genotyping in race horses.

Proteomic markers:

A single study (Bouwman et al., 2010) has elucidated the differential expression of proteins in the skeletal muscle of trained and untrained equines though 2D gel electrophoresis and mass spectroscopic analysis. The differential expression of 16 proteins exhibited structural changes towards higher oxidative capacity, higher capacity for long chain fatty acids, and to store more energy in the form of glycogen. In normal exercise training, epithelial keratin-1 was highly down regulated (20 fold decrease) after training. C-protein phosphatase-1 regulatory subunit 9B, troponin T, myoglobin, UTP-Glucose-1-phosphate uridylyltransferase 2 (matched to bovine), alpha crystalline B chain (pig), aspartate aminotransferase mitochondrial (horse) were the ones found upregulated. In case of the intensified exercise, seven proteins were over expressed, of which only alpha-1 antitrypsin was not found to be increased after normal training. This protein is postulated to be considered as a marker for overtraining in the horses (Bouwman et al., 2010). The increased expression of the complex of troponin T , myoglobin and mitochondrial aspartate aminotransferase I increases oxygen transfer in the muscle. Troponin T is a thin filament protein and has both slow and fast isoforms. The training is supposed to result in an abundance of slow isoform which leads to increased oxidative capacity (Bouwman et al., 2010), a preferred adaptation in the exercising horses. Alpha crystalline B increased 3.2 fold during training and facilitates refolding of the non native proteins. Higher expression of the mitochondrial aspartate aminotransferase (2.1 fold) leads to increasing uptake of long chain saturated as well as unsaturated fatty acids by the metabolic cells thereby sparing glucose and oxidizing lipids for providing energy during exercise. (Bouwman et al., 2010).

CONCLUSIONS

Physiological responses and fitness indices have strong relationship with the performance of horses under exercise of various intensities. Long term adaptations to exercise as a result of training induces both phenotypic and genetic adaptations. The generation of large amount of horse transcriptome data through high throughput sequencing techniques and its analysis has facilitated identification of several candidate and functional genes transcripts which are now being established as definitive markers of exercise performance in equines. While genotyping of a few genes such as MSTN and CKM is being employed for identification and selection of elite performance equines for different category of exercise, many more potential markers are yet to be established and exploited commercially in the horse breeding and race industry. Future research on these lines in the different horse breeds (other than the widely studied thoroughbreds and standardbreds), that have evolved and adapted to extreme climate zones would help enhance our understanding of the physiological and molecular principles of exercising performance and adaptation in horses. Incorporation of genomic signatures in horse breeding and training programs would help in development of healthy, elite and high performance equines.

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