The role of HIF1α, VEGFα and MCT4 on performance in racing horses

A. Behairy [1], Hussein A. Heshmat [1], Hussein Abaza[2]

[1]Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.
[2] Dean of Faculty of Physical Education, Banha University, Egypt
E-mail: amanybehairy25688@gmail.com

Abstract: For several decades, equine physical performance has been a subject of research; just some studies have been directed for molecular genetic analyses. To date, examines in people have reported several genes related with elite athletic performance, while thinks about in horses are uncommon. In our research we aimed to illustrate the role of certain genes which linked with exercise and training, and have been revealed to be functionally relevant for early performance assessment of elite athletic horses. Ten racing horses (5 Arabian and 5 Thoroughbred horses) were selected from equestrian clubs in Cairo Governorate. Firstly, physical parameters included (heart rate and temperature) were detected. Then, the blood samples obtained from both groups before and instantly after training from trained horses. Quantitative real time PCR used to measure **HIF1α, VEGFα and MCT4** gene expression. Our results reported a considerable increase in **HIF1α, VEGFα and MCT4** gene expression after training in trained horses. The results determined the pivotal role of **HIF1α, VEGFα and MCT4** in exercise and their important effect on equine performance during hypoxic training. In conclusion, this study interprets underlying molecular responses to exercise and training adaptations in equine blood and how these responses affect on system-wide physiological performance. Biology of the HIF1 pathway and other related genes affecting horse performance have seen great interest. Despite late great evolution in understanding the atomic instruments of the HIF pathway because of its activity, several important questions still needed to be answered.
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Key words: HIF1α, VEGFα, MCT4, Arabian, Thoroughbreds, Racing horses.

1- Introduction:

Equine muscles design and changes were subjected long ago to serious investigations, especially in its molecular and cellular particles **(Rivero and Hill, 2016)**. They also added that the horse fitness might be attributed to exceptional physiological and anatomical changes of body compartments related to exercise. As the horse posses large muscles to be able to run faster or can endure as pulling and carrying heavy things **(Schröder et al., 2011).** The best performing horses characterized by huge muscles, low body fat percent, together with a large percent of white or red muscle types **(Kearns et al., 2002)**.

Oxygen is necessary for production of energy in electron transport system (**West, 2004**). Oxygen lack lead to vasodilation to preserve O_2 hand over to the acting muscles, while is affected by the exercise grade **(Casey and Joyner, 2012**). The greater oxygen delivery might increase performance in racehorse (**Erickson et al., 1994**). **Millet et al. (2012**) noted that the highest grade of hypoxia inhibits performance compared with normal oxygen supply.

Eiken and Tesch, (1984) reported a decreased performance due to hypoxia as a result of H+ collection from O_2 lack. This will hinder central nervous system (CNS) and performance (**Amann and Kayser, 2009**). Hence, central fatigue development, affecting motor output, elevated cerebral metabolism and decline performance, and the better power output resulted from increased O_2 supply, indicating the sharing of CNS in performance decrease due to low oxygen supply (**Amann et al., 2007**).

In most living organisms the requirement for cellular oxygen homeostasis has created a functional and rapid molecular response system that detects hypoxia, prompting the induction of several adaptive genes that encourage expanded oxygen supply and support anaerobic ATP generation **(Taylor, 2008)**. There are certain confirmations that exercising in hypoxia influences muscular functions (**Lundby et al., 2009**) and an extensive number of genes intervened by hypoxia-inducible factors (HIFs) (**Semenza et al., 2006**). HIF-1· is known to be a basic factor for prompting lines of genes supporting cell survival such as vasomotor regulation (endothelin-1 and heme oxygenase-1), glucose metabolism (glycolytic enzymes and glucose transporters), anemia control (transferrin and erythropoietin) and angiogenic growth (vascular endothelial growth factor) **(Rosenberger et al., 2002)**. This study explored the possible molecular mechanism involved in improving of performance in race horses, and to prove this we performed gene expression of Hypoxia inducible

factor 1α (HIF1α), Vascular endothelial growth factor α (VEGFα) and Monocarboxylate transporter 4 (MCT4).

2- Materials and Methods:

2.1 Animals and Experimental Designs:

Totally, ten (10) male horses were chosen from equestrian clubs in Cairo Governorate. Horses were separated into 2 groups of 5 regularly trained Arabian horses and other 5 regularly trained Thoroughbred animals, stayed in separated boxes. Horses were traditionally fed with hay with a blend of cereals (oats and barley) provided three times each day (08:00, 12:00 and 20:00) and given water ad libitum. The groups of trained horses underwent training 4 days a week. The training courses were held in the morning from 7- 9 am. **The training include**: 100m…walking, then 100-200m…trotting, followed by 200-300m…narrow canter, then 400-600m…wide canter finally 100-200m…galloping.

2.1.1 Physical measurements:

Clinical studies of horses were performed earlier and following exercise, these clinical examinations required the estimation of rectal temperature (T) and the examination of heart rate (HR). Body temperature examined by thermometer and heart rate recorded by auscultation.

2.2 Blood Samples:

Blood samples were gathered by jugular venipuncture from trained horses before and instantly following the exercise in both Arabian and Thoroughbred horses. Samples were aspirated into 10 ml syringes and promptly transferred into sterile K3 EDTA tubes and kept quickly in ice boxes, transferred promptly to biochemistry department, Kasr Al. Ainy, Faculty of Medicine, Cairo University for Real time PCR for HIF1α, VEGFα, MCT4 genes.

2.3 Molecular Analysis:

2.3.1 HIF1α, VEGFα and MCT4 Real-time Polymerase Chain Reaction

2.3.1.1 Isolation of PBMN cells:

Firstly, the mononuclear cells isolated from whole blood through using of Ficoll-Paque Premium **(Biochrom, Berlin, Germany).**

2.3.1.2 Total RNA Extraction:

Then, the total RNA was extracted from cell pellet utilizing **SV Total RNA Isolation System kit, Promega, Madison VVI, USA (Cat. # Z3100).** It was intended for isolation of total intranuclear RNA from fresh, entire blood treated anticoagulated with EDTA.

2.3.1.3 Quantitation and Storage of Total RNA

To decide the concentration and purity of Total RNA, absorbance at 260 nm and 280 nm was measured in a spectrophotometer.

Concentration of RNA sample (µg/ml) = 40 X A 260 X dilution factor

2.3.1.4 Reverse Transcription:

The extracted RNA was reverse transcribed into cDNA using: **AMV Reverse Transcriptase, Promega, and Madison. VVI, USA (Catalog No.: M5101).** Total volume of the master mix was **19 µl** for each sample. This was added to the **31 µl** RNA-primer mixture resulting in **50 µl** of cDNA.

2.3.1.5 Real-time Polymerase Chain Reaction

A real time- PCR reaction mixture was 50µl. The following mixture was prepared in each optical tube (25 µl SYBR Green Mix (2x), 0.5 µl kidney cDNA, 2 µl primer pair mix (5 pmol/ µl each primer), 22.5 µl H_2O). Primers were designed from various exons of each gene to avoid amplification of genomic DNA. The sequences of the primers utilized in the study are recorded in **Table (1)**. Because data on horse MCT4 was not available, primers were planned using homologous regions of the MCT4 of different species (**Mykkänen et al., 2011**). The sequence found in the horse transcriptome resembling the MCT4 cDNA sequence in other species is, respectively, 89% and 87% homologous to cattle and putative dog MCT4 cDNA sequences. Similarly, the candidate for horse MCT4 protein is 92% and 91% symmetrical to the cow and putative dog sequences, respectively (**Mykkänen, 2011**).

Table (1): Sequence of the primers used for real-time PCR

Primer sequence	Reference
Forward 5'- atctgacctgccgcctggag -3' Reverse 5'- cgatgcctgcttcaccaccttc -3'	GAPDH **(Toussaint et al., 2012)**
Forward:5`- ctcaaatgcaagaacctgctc -3` Reverse: 5`- ttccataccatcttttgtcactg -3`	HIF1-α **(Toussaint et al., 2012)**
Forward 5'- tggcagaaggagagcataaaa -3' Reverse 5'- actcgatctcatcggggtact -3'	VEGF-α **(Toussaint et al., 2012)**
Forward 5'- cagcccttaggtgcctctct -3' Reverse 5'- gccacagcgtagatcacaaa -3'	1 MCT4 **(Mykkänen et al.,2011)**
Forward 5'- gctgctcaactgctgtgtgt -3' Reverse 5'- ggcttggcttcatcacagat -3'	2 MCT4 **(Mykkänen et al., 2011)**

The thermal PCR conditions used were (50°C 2 min., 1 cycle, 95°C 10 min., 1 cycle, finally, 95°C 15 sec and 60°C 30 sec. for 40 cycles). At the end of a qPCR running with SYBR Green chemistry, the relative quantification was estimated using Applied Biosystem soft ware.

2.4 Statistical Analysis:

Data were analyzed with the Statistical Package for the Social Sciences (SPSS), version 21.0 and expressed as mean ± standard error of means (S.E.M). Paired comparison t-test was utilized to test the horse blood samples collected at rest and immediately after exercise for any critical difference. Differences between groups were assumed to be significant at P<.05. (**SPSS, 21.0 software, 2012**).

3- Results:

Our data reveled significant rise in temperature in Arabian horses after training (118.33±1.20) than before (45.33±1.33) ***(p<0.01)*** as showed in **table (2)**. Moreover by examining heart rate, the examination revealed a critical rise in heart rate after training (38.77±.15) comparing with before training (37.27±.145) ***(p<0.05)***.

Table (2): Represents Mean values (±SE) and paired comparison T test of physical parameters of Arabian horses at rest and after exercise:

	Before	After	t-test
Heart rate (b/m)	45.33±1.33	118.33±1.20	-73.00**
Temperature (°c)	37.27±.145	38.77±.15	-8.66*

Mean values (±SE) **p < 0.01(highly significant difference) *p < 0.05(significant difference)
ns: non significant

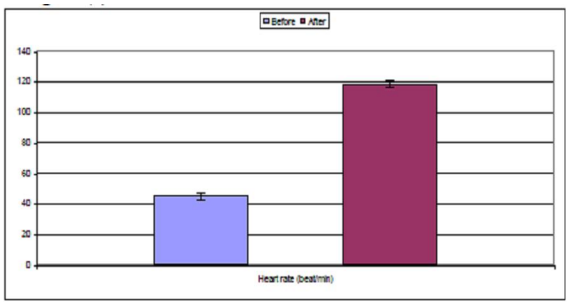

Figure (1): Heart rate of Arabian horses:

As showed in **table (3),** a great rise in heart rate in Thoroughbred horses from (38.33±1.20) before exercise to (81.33±2.67) after exercise was found ***(p<0.01)***. Moreover, the temperature increased from (37.63±.033) before exercise to (38.30±.153) after exercise ***(p<0.05)***.

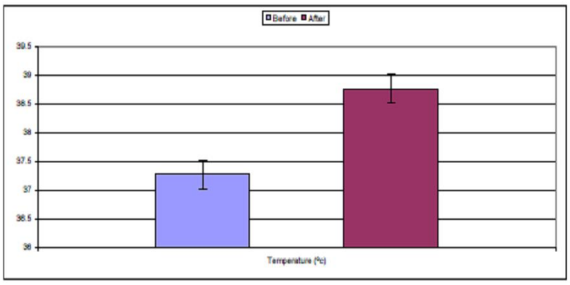

Figure (2): Temperature of Arabian horses:

Table (3): Represents Mean values (±SE) and paired comparison T test of physical parameters of Thoroughbred horses at rest and after exercise:

	Before	After	t-test
Heart rate (b/m)	38.33±1.20	81.33±2.67	-13.78**
Temperature (°c)	37.63±.033	38.30±.153	-4.71*

Mean values (±SE) **p < 0.01(highly significant difference) *p < 0.05 (significant difference)
ns: non significant

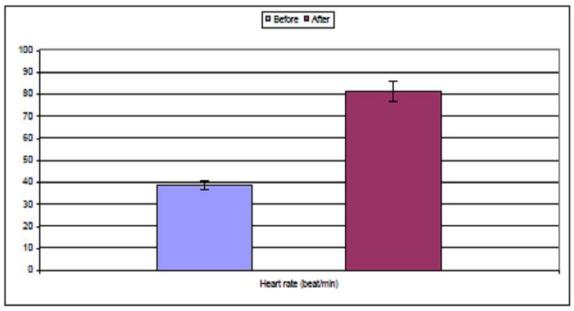

Figure (3): Heart rate in Thoroughbred horses:

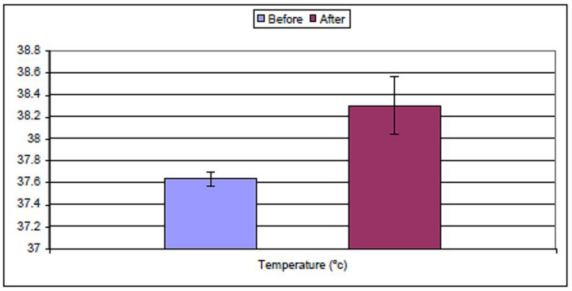

Figure (4): Temperature in Thoroughbred horses:

HIF1 α, VEGFα and MCT4 gene expression revealed a high significant rise in Thoroughbred horses between the rest (1.03±0.05) (1.01±0.02) (1.03±0.04) and the post exercise period (12.5±1.48) (19.47±2.39) (1.96±0.07) ***(p<0.01)*** respectively as showed in **table (4)**. The increase of VEGFα is correlated to the increase of HIF1α (.937**) and also the increase of MCT4 is correlated with increase of HIF1α (.893**) and VEGFα (.681**).

Table (4): Represents Mean values (±SE) and paired comparison T test of HIF1α, VEGFα and MCT4 gene expression in Thoroughbred horses at rest and after training:

	Group 1) Thoroughbred horses)		
	Before	**After**	**T-Test**
HIF1α	1.03±0.05	12.5±1.48	-13.71**
VEGFα	1.01±0.02	19.47±2.39	-13.30**
MCT4	1.03±0.04	1.96±0.07	-16.70**

Mean values (±SE) **p < 0.01 (highly significant difference) *p < 0.05(significant difference) ns: non significant

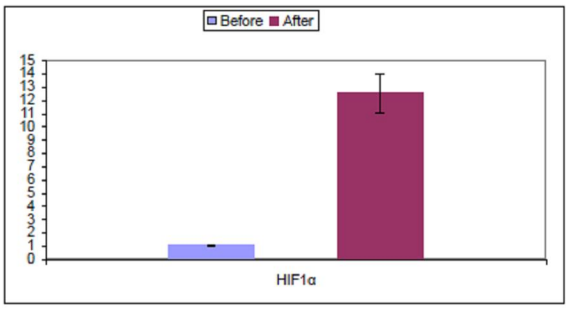

Figure 5: HIF1α gene expression in Thoroughbred horses:

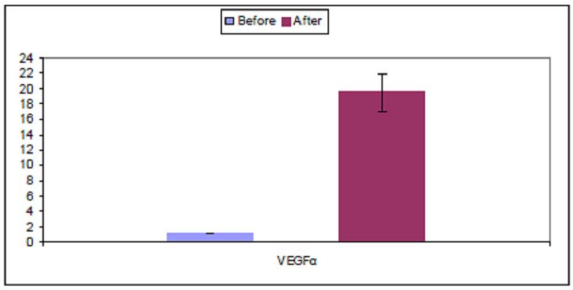

Figure 6: VEGFα gene expression in Thoroughbred horses:

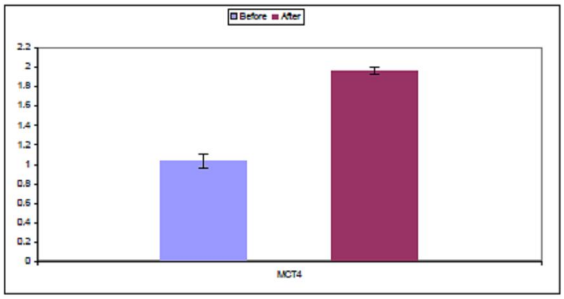

Figure 7: MCT4 gene expression in Thoroughbred horses:

The findings represented in **table (5)** revealed great increase in gene expression of HIF1α and VEGFα from (1.01±0.02) (1.03±0.04) at rest to (9.07±3.03) (13.8±3.4) ***(p<0.05)*** after training in Arabian horses respectively. The increase of HIF1α and VEGFα correlated together (.952**). In contrast, there was non significant change in MCT4 gene expression before (1.02±0.02) and after training (1.28±0.42). MCT4 correlated with HIF1α (.996**) and VEGFα (.887**).

Table (5): Represents Mean values (±SE) and paired comparison T test of HIF1α, VEGFα and MCT4 gene expression in Arabian horses at rest and after training:

	(Group 2) Arabian horses		
	Before	**After**	**T-Test**
HIF1α	1.01±0.02	9.07±3.03	-4.59*
VEGFα	1.03±0.04	13.8±3.4	-6.57*
MCT4	1.02±0.02	1.28±0.42	-1.04

Mean values (±SE) **p < 0.01 (highly significant difference) *p < 0.05 (significant difference); ns: non significant

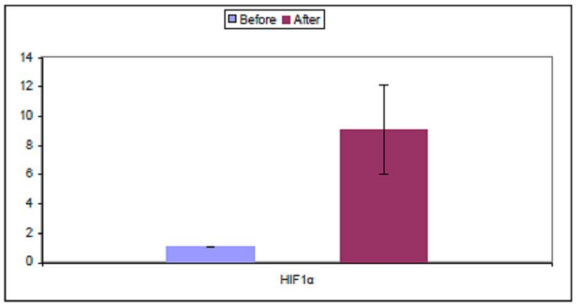

Figure 8: HIF1α gene expression in Arabian horses:

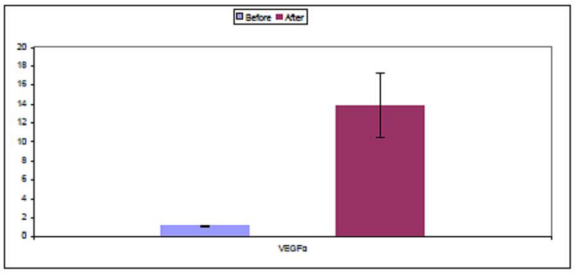

Figure 9: VEGFα gene expression in Arabian horses.

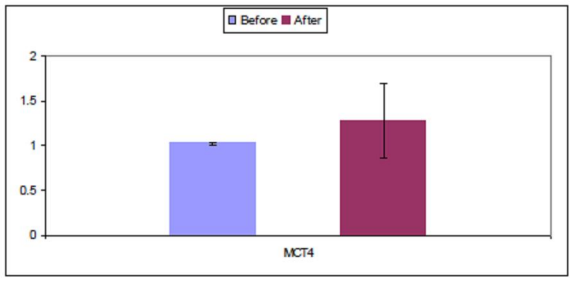

Figure 10: MCT4 gene expression in Arabian horses.

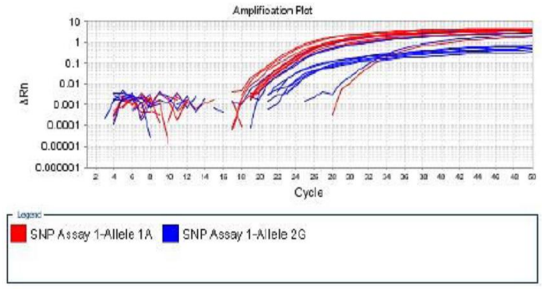

Figure 11: Amplification curve for HIF1α.

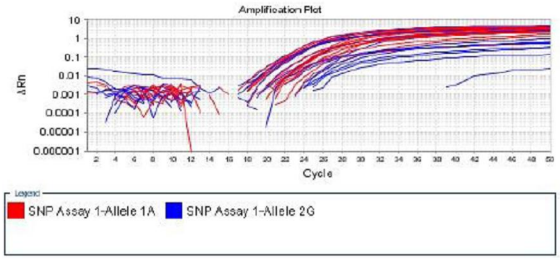

Figure 12: Amplification curve for VEGFα.

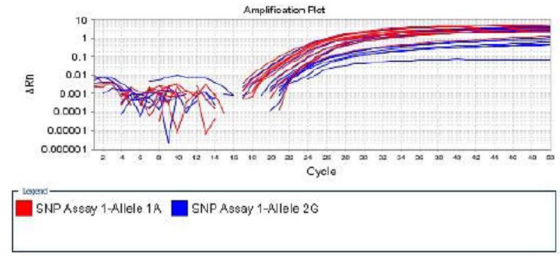

Figure 13: Amplification curve for MCT4.

4-Discussion:

Our data revealed an important increase in temperature in both Arabian and Thoroughbred horses after training than before. Consistent with **Janicki *et al*. (2013)** who demonstrated a large elevation in the rectal temperature promptly after the exercise. It is assumed that horses have a great metabolic capacity and a little surface area for dissipation of heat. When horses exercise, about 80% moves toward becoming warmth while the remaining 20% of the metabolism in the muscle cells is utilized for work (**Brody, 1945**). **Charkoudian, (2010)** added that skin blood flow increases during internal body heating trying to transfer the heat from body core to the surface of the skin, and sweating begins if vasodilatation is not sufficient.

A great rise in heart rate after exercise than before exercise in both Arabian and Thoroughbred horses. These results consistent with **Vincent et al. (2006)** who reported that HR increases greatly from rest to strenuous exercise reaching maximal estimations in the district of 210–240 beats/min. Heart rate can return back to 64 bpm within 20 minutes post practice in well trained horses **(Munsters et al., 2014)**. HR increased during each exercise session with no distinction between horses **(Wallsten et al., 2012)**. During exercise, the muscles demand increased blood flow and this is carried out by increasing in heart rate and stroke volume. Stroke volume is correlated with heart size and horses have large hearts compared with different species, with values for heart mass of approximately 0.9% of body mass in untrained horses **(Poole, 2004)**. Heart size and accordingly SV also rise further with training **(Buhl et al., 2005)**. Recently, **Kang and Park (2017)** reported that great amount and grade of exercise increase the heart size and the heart becomes more adaptive.

Interestingly, variations in HR during exercise are a measure of physical stress, HRR (heart rate recovery) recorded under standardised conditions may be interpreted as a reasonable measure for current capacity of the human body's to react to exercise stress **(Borresen and Lambert, 2007)**, and also for horses **(Bitschnau et al., 2010)**. **Hada et al. (2006)** observed that HRR after exercise was enhanced with training in Thoroughbred racehorses. If the capacity of horse is improved, the heart rate diminishes in the assigned exercise speed or exercise grade and reduces quickly even after completing activity **(Kang and Park, 2017)**.

The hereditary qualities of elite physical performance in horses are complex involving a great number of organ systems and metabolic pathways, and are likely polygenic with the combined effect of hundreds of genetic variants **(Schröder et al., 2011)**. As concerns HIF1α, we observed exceedingly severe increase in HIF1α after exercise than before exercise in both Arabian and Thoroughbred horses. HIF-1a coordinates an appropriate metabolic response of the cell to hypoxia **(Halestrap and Wilson, 2012)**. The increased HIF-1 activity alters skeletal muscle metabolism and prompts decreased muscle pH and expanded serum lactate collection in react to exercise **(Lindholm and Rundqvist, 2016)**.

HIF1 is a heterodimer made out of an oxygen-regulated α-subunit and a constitutively expressed β-subunit. In general the plenitude of α-subunits is basically managed by a family of prolyl hydroxylases (PHD). In normoxia, PHD is initiated and guides the degradation of the α-subunit by the ubiquitin-proteasome pathway **(Jaakkola et al., 2001)**. Under hypoxia, PHD activity diminishes, which prompts the stabilization and translocation to the nucleus of the α-subunits which heterodimerize with the β-subunit. This dimer perceives the hypoxia response elements (HREs) to start target gene expression. After a time of exercise training, the initiation of HIF-1 and its target genes respond to intensive exercise may be decreased **(Lindholm and Rundqvist, 2016)**.

Practicing skeletal muscle experiences severe and repetitive oxygen stress during endurance training and glycolysis and angiogenesis are up- managed by HIF1A responding to hypoxia **(Semenza, 1998)**. In our research the increase in HIF1α gene expression in Arabian horses is less than observed in Thoroughbred horses. **Lunde et al. (2011)** observed that overexpression of HIF-1α has been correlated with a fast muscle fiber type. The loss of skeletal muscle HIF-1 may be useful for oxidative capacity and endurance performance **(Lindholm and Rundqvist, 2016)**. Mice with a skeletal muscle- particular deletion of HIF-1α perform better in swimming and running endurance trials and show, several of the features related with a trained muscle particularly in connection with mitochondrial characteristics such as enzyme activities of citrate synthase and 3hydroxyacyl-CoA dehydrogenase **(Mason et al., 2007)**. Hypoxia-inducible factor-1 controls mitochondrial oxygen utilization in cells during hypoxia through expanded expression of (PDK-1) (pyruvate dehydrogenase kinase 1) **(Kim et al., 2006)**. In the knockout mice, reduction of HIF-1α activity prompted lessened PDK-1 (pyruvate dehydrogense kinase 1) levels before and instantly after an acute bout of exercise which could clarify the enhanced mitochondrial action and the diminished lactate accumulation seen following exercise **(Mason et al., 2004, 2007)**. What's more, In Thoroughbred horses (as in humans), the VO_2max is generally restricted by oxygen supply to the mitochondria rather than by mitochondrial oxidative capacity **(Katz et al., 2005)**, and arterial hypoxemia stimulated by exercise can occur when the increased oxygen requirements cannot be achieved during maximal exercise **(Dempsey and Wagner, 1999)**.

Conversely, **Eynon et al. (2010)** reported that sprinting performance was affected by an interaction of the HIF1A P/P and the ACTN3 R/R genotypes whereas the HIF1A genotype distribution among sprinters, endurance athletes and controls did not differ.

Our data observed highly significant rise in VEGFα gene expression after exercise in both Arabian and Thoroughbred horses. The findings agree with **Park et al. (2012)** who revealed that equine VEGFα transcript elevated significantly in response to exercise. The expression level of VEGFα was the highest following 60 min of exercise and then gradually diminished after up to 120 min. But the expression level of 120 min was somewhat higher compared with level at 0 min (before exercise). Equine VEGFα was observed to be ubiquitously expressed but managed by exercise in blood leukocytes, proposing that horse VEGFα expression in leukocytes can be utilized as a vital sign for recovery after exercise **(Song et al., 2014)**.

One explanation for how VEGFα expression is upregulated and achieved the most extreme level after 60 min of exercise is that intensive exercise may create the hypoxia condition in leukocytes, which triggers hypoxia inducible factor-1α (HIF-1α) activation. Activated HIF-1α will prompt the transcriptional activation of VEGFα by binding to HIF-responsive elements which are located in the promoter region of VEGFα **(Jiang et al., 1996)**. In addition, the circulating VEGFα encourages mobilization of monocytes into injured areas after exercise, and boosts wound healing by stimulating endothelial cell proliferation and extracellular matrix changes as process of recovery **(Richardson et al., 1999)**. Interestingly, **Richardson et al. (2000)** observed that the raise in VEGF mRNA stimulated by acute exercise is lessened after a time of endurance training.

In our study, we found a significant correlation between HIF1α and VEGFα throughout the training period and this consistent with **(Song et al., 2014)** who inspected equine HIF-1α expression in blood leukocytes after exercise and reveal the causal connection between HIF-1α and VEGFα expression. Hypoxic HIF-1 activation stimulates its binding to HREs, (hypoxia response elements) leading to motivation of genes which increase accessibility of oxygen, such as EPO and VEGF **(Semenza, 2000)**.

Our results demonstrated a statistically important increase in MCT4 after exercise in Thoroughbred horses. These results affirm the previous study of **Kitaoka et al. (2011)** who evidenced the basic function of MCTs for physiological capacity where the amount of MCTs in horse muscle has been raised by training. **Green et al. (2002)** demonstrated that a single exercise session in humans performed at 60% of VO_2max for 6 hours generated elevations in MCT1 and MCT4 throughout the 6 days after exercise. The race-fit horses indicated higher MCT4 expression than moderately trained horses **(Koho, 2011)**.

During intense exercise, aerobic ATP production is not sufficient for the energy demands of muscle, and so the metabolism shifts towards the anaerobic pathway of ATP production, leading to the collection of lactate and protons in the muscles and blood **(Mykkänen, 2011)**. The high intensity interval-type training with gradually increasing accumulation of lactic acid could be a trigger for increasing MCT4 expression in the muscle cells. A similar impact might be accomplished by hypoxia **(Ullah et al., 2006)**. The increase in intracellular lactate during high-intensity exercise in horses has been distinguished as a reason for fatigue **(Rivero and Piercy, 2014)**. The buffers and transfer of protons and lactate anions outside the

cell are needed to prevent the muscle acidosis. The buffering capacity of equine muscle is higher than in other different species, and MCTs are responsible for most proton efflux. Two MCT isoforms, MCT1 and MCT4, are both expressed in horse muscles **(Koho et al., 2006)**. Lactate formed in glycolytic fibers could be removed via MCT4 while reached oxidative fibers through MCT1, being oxidised by subsarcolemmal mitochondria as a substrate for ATP resynthesis **(Rivero and Hill, 2016)**. Acute incremental exercise causes a transient increments in MCT1 and MCT4 transcript expression and protein content **(Kitaoka et al., 2013)**. The lactate may reach the circulation and be metabolized in other skeletal muscles, heart, and liver if it cannot be transferred to the oxidative muscle fibers in the neighborhood **(Gladden, 2004)**. This adaptive response allows the increased lactic acid produced during hypoxia to be lost from the cell rapidly **(Ullah et al., 2006)**.

Furthermore, horse skeletal muscles have a great mitochondrial volume which allows a higher entire animal aerobic capacity, and also vast intramuscular stores of energy substrates (glycogen specifically). Certain adaptations as high buffer and lactate transport capacities can be stimulated with training trying to save muscles against fatigue during anaerobic exercise **(Rivero and Hill, 2016)**. The mass of active muscle and the intensity of the activation of these muscle determine the net arrival of lactic acid from muscle to blood **(Poortmans et al., 1978)**. There will be diverse variations relying upon fiber types, effectors of membrane lactate transport, blood flow and the thermal conditions affecting its distribution **(Billat et al., 2003)**. The increased blood epinephrine level will build the net yield of lactic acid by the working muscles **(Stainsby and Brooks, 1990)**.

High intensity training (HIT) is required to increase and also to keep up MCT4 protein content **(Kitaoka et al., 2010a)**. It might suggest that lactate expulsion by MCT4 is more critical for maximal exercise performance and training adaptation than MCT1 **(Kitaoka et al., 2010a)**. It is already announced a critical connection between MCT4 protein content and maximal exercise performance in equines **(Kitaoka et al., 2010b)**.

Our data indicated that the increase in MCT4 gene expression is higher in Thoroughbred than Arabian horses. Consistent with **Kitaoka et al. (2013)** who previously demonstrated in Thoroughbred horses that acute exercise and training increase the expression of MCT1 and MCT4 proteins. **Kitaoka et al. (2014**) added that during exercise, Thoroughbred horses with higher muscle lactate creation are provided with higher MCT4 protein content where the efflux of lactate via these MCT4 may be mostly important for racehorses because of their high glycolytic and oxidative capacity.

In humans, 6 weeks of sprint-interval training increased content of MCT1 and MCT4 proteins and after 6 weeks of detraining this increase came back to the baseline level **(Burgomaster et al., 2007)**. The study of **Kitaoka et al. (2010a**) demonstrated that high intensity training (HIT) for18 weeks increased MCT1 and MCT4 protein contents in skeletal muscles of Thoroughbred horses. The decrease of MCT4 protein content after detraining reduced exercise performance, even though MCT1 protein content and oxidation capabilities were maintained in Thoroughbred horses **(Kitaoka et al., 2011)**. **Koho et al. (2006)** reported that older and more trained animals have more MCT4 but not MCT1 expression in their muscle compared to untrained young individuals **(Koho et al. 2006)**. Equine skeletal muscles contain large stores of glycogen that are promptly accessible during exercise. After intensive exercise, muscle glycogen concentration diminishes by up to 50%, and therefore a large amount of lactate is produced **(Hyyppa et al., 1997)**. Muscle glycogen utilization and subsequent muscle lactate accumulation occurs early during exercise **(Kitaoka et al., 2014)**.

As concerns with Arabian horses **Dubouchaud et al. (2000)** observed that after training, the content of MCT1 increased fundamentally in most of the muscle compartments, whereas the overall muscle preparations content of MCT4 was not in a general sense modified. These observations were related with a 100% increase in the sarcolemmal GLUT-4 content and reflect membrane adaptations to endurance training that were not confined to lactate transporter isoforms. Interindividual differences in MCT4 expression and the absence of MCT4 in the mitochondria recommend that MCT4 is a constitutive sarcolemmal lactate transporter isoform and is less delicate to endurance training than the MCT1 isoform **(Dubouchaud et al., 2000)**.

The increased aerobic capacity of skeletal muscle following training occurs concurrently with a critical decrease in the net rate of muscle glycogenolysis during prolonged submaximal exercise **(Geor et al.,1999)**, postponing the beginning of fatigue by saving muscle glycogen and increasing fat oxidation as a wellspring of energy. The oxidation of fatty acids is critical for Arabian horses that perform long and submaximal exercises to deliver required energy for an extended period of time **(Regatieri et al., 2016)**. In contrast, **Bonen et al. (2000)** reported that lactate transport can be expanded when muscle action is incessantly expanded or by endurance training.

The other direction in MCT studies is that **Kitaoka et al. (2010b)** neglected to demonstrate an increase in either MCT1 or MCT4 expression in the gluteus medius of Thoroughbred racehorses after seven weeks of intensive training. Regular (3–4 sessions per week) high-intensity (>85% VO_2max) exercise improves anaerobic capacity in horse skeletal muscles, but this is not reflected in blood lactate concentrations during maximal, comprehensive exertion **(Rivero et al., 2007)**. In human subjects, after an acute period of high intensity exercise, the membrane expression of MCT1 and MCT4 have diminished by roughly 20% **(Bishop et al., 2007)**. There might be a species difference in the transcriptional and post-transcriptional control of MCT1 expression **(Mykkänen, 2011)**.

In our study, we watched critical direct connections amongst MCT4 and HIF1α. The MCT4 promoter joins those of other glycolytic enzymes that are controlled by HIF-1α including enolase 1, aldolase A, lactate dehydrogenase A, and glucose transporter 1 **(Semenza, 2001)**. A line of evidence recently accumulated indicates that HIF-1 not only activates glycolytic pathways but that it also suppresses mitochondrial work and is responsible for a lowering in mitochondrial action and oxygen utilization **(Lindholm and Rundqvist, 2016)**. The pivotal role of MCT4 as a lactate exporter is supported by the up-regulation of its gene by hypoxia-inducible transcription factor (HIF)-1 **(Ullah et al., 2006)**.

5-Conclusions:

The physiological responses and wellness records have solid association with the performance of horses under exercise of various intensities. The instant and long- term adjustments in the physiological functions induced by exercise in the horses are brought about at the molecular level by transient changes in the gene transcriptions. Gene expression of a few genes such as HIF1α, VEGFα and MCT4 is being utilized for distinguishing and selection of elite performance equines for various categories of exercise.

Brief times of hypoxic training was adequate for inducing a significant upregulation of HIF-1α and hence prompts a downstream stimulation of HIF-1α dependent pathway.

MCT4 assumes a major role during maximal exercise in horses, expressed in glycolytic tissues and mediates lactic acid efflux from the cells. Horse VEGFα transcript elevated significantly in react to exercise. Thus, the precise relationship among exercise, the HIF-1α pathway including VEGFα, MCT4 needs further study.

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