The integration action of stem cells and performance genes in selection of racing horses

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Abstract: Although many recent researches tried to devise new techniques for fitness assessment, more techniques are still needed for routine monitoring of horses in training that lead to fitter horses with lower rates of injury and fatigue. Gene characterization is an important feature for genome annotation. Until now few studies have revealed performance genes in elite athletic performance in racing horses. In our study we tried to find a new tool for selection of racing Arabian horses by breeders and help trainers adapt training and racing programs according to horses´s genetic makeup. In addition, the study aimed to determine the effect of exercise and/or training on certain performance genes and hematopoietic stem cells (CD $^{34+}$) in racing horses. Six Arabian horses were chosen from equestrian clubs in Cairo Governorate, characterized as three trained and three untrained horses. Firstly, anthropometric measurement and physical parameters were detected. Then, Peripheral blood samples collected from both groups at rest time and after training from trained Arabian horses. Genomic DNA was extracted from peripheral blood samples and genotyping analyses were performed by PCR methods. Manual counting by using hemocytometer used to assess mobilized hematopoietic stem cells (CD $^{34+}$). Quantitative real time PCR used to measure ACE and ACTININ 3 gene expression. Our results indicated that there was a significant increase in total RNA in trained Arabian horses at rest and after exercise. Furthermore, there was a highly significant increase in ACE and ACTININ 3 gene expression before and after training in trained horses. No significant difference between genotype in trained and untrained horses. In addition, mobilized stem cells increased by training at rest and after exercise. In conclusion, there is sufficient scientific evidence for usage of ACE and ACTININ 3 gene expression as biomarkers of superior athletic performance in racing horses. Our study shed light on usage total RNA as a tool for assessment athletic performance and a good genetic indicator for individual variation among sporting horses. Moreover, the study revealed the effect of exercise on mobilizing stem cells and thereby enhancing tissue repair mechanisms and is a marker of elite horse. Both increase of stem cells and performance gene expression revealed an integrative action in racing horses selection.

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1- Introduction:

Physical fitness is operationalized as several measurable health-related phenotypes including mainly cardiorespiratory fitness and muscle performance/function **(Garber *et al*., 2011).** Regular exercise induces several beneficial changes in organs and biological pathways that lead to better physical performance and improved health outcomes **(Kim *et al*., 2015).**

Level of fitness or exercise tolerance of a horse is assessed by physical examination of heart rates and respiratory rates **(Bashir and Rasedee, 2009).** Good conformation increases the probability that a horse can perform the functions characteristic of its breed for an extended period of time without becoming unsound, but is not a guarantee. “Faulty” conformation may impair some activities and may predispose a horse to unsoundness (e.g., injury, lameness **(Wright and Rietveld, 2007).**

Treadmill testing is a valuable diagnostic tool for horses with decreased athletic ability because it facilitates examination of the respiratory system during exercise **(Davidson *et al*., 2011)**. A fitness test, which is preferably conducted on a treadmill under standardized conditions, involves working the horse during intervals of 90 seconds at progressively higher intensities. The heart rate is determined continually and during each work level a blood sample is taken for the determination of the blood lactate concentration: The lower the heart rate and the lower the lactate concentration for a defined workload, the fitter the horse **(Weishaupt *et al*., 2006).**

On the treadmill, a great range of measurements are possible during exercise, and testing conditions may also easily be standardized. Despite this, treadmill testing does not reproduce the natural environment of the horse, and some authors have shown that the energy expenditure during exercise on the treadmill is quantitatively different from that during exercise under field conditions **(Barrey *et al*., 1993).**

As the intensity of exercise is increased however, the rate of lactate entry into the blood from muscle eventually exceeds its rate of removal from the blood, and blood lactate concentrations increase above resting levels. From this point on, lactate levels continue to increase as the rate of work increases, until the point of exhaustion. The point at which the concentration of lactate in the blood begins to increase above resting levels is referred to as the lactate threshold **(Wilmore and Costill, 1994).**

In addition, **Evans and Polglaze, (1994)** observed that the blood lactate concentration after maximal exercise to fatigue does not change with submaximal exercise training, and so is not a useful marker of fitness.

The study of **Kargarfard *et al.* (2011**) proved that exercise in the high-polluted area led to significant decrease in the mean Vo_2 max and significant increase in the mean serum lactate level. The reduced aerobic physical activity can be attributed to the increase in COHb, so the individual would enter earlier to the anaerobic system. This may result in decreased anaerobic threshold and in turn in accumulative lactate production, as well as in reducing maximum oxygen consumption.

The Conconi test (CT) is one of the field tests commonly used in running events. This test was created to determine the anaerobic threshold (AT) by forming a relationship between running speed and heart rate (HR) during an incremental test. HR relationship has been described as linear from low-to-sub maximal speeds and curvilinear from sub maximal-to-maximal speeds. This transition from the linear to the curvilinear phase coincided with the beginning of blood lactate accumulation **(Celik *et al*., 2005).**

Predictive genetic tests may be a tool for selection by breeders and could help trainers adapt training and racing protocols according to the specific genetic makeup of the horse as is already happening in human professional sports **(Dennis, 2005).** The horse is an optimal model organism for studying the genomic response to exercise-induced stress, due to its natural aptitude for athletic performance and the relative homogeneity of its genetic and environmental backgrounds **(Capomaccio *et al.*, 2013).** Specifically, two genes have been extensively studied for their association with athletic ability, namely the ACE and ACTN3 genes **(Tucker *et al*., 2013).**

Different frequency of ACE I/D and ACTN3 R577X polymorphisms have been reported in different populations worldwide, and human body is a complicated combination and organization which is influenced by multiple factors as race, lifestyle, environment, and nutrition as well as genetic and training **(Ooi *et al*., 2015).** I and D alleles of the ACE gene are determinants of endurance and power, respectively and that the R and the X alleles of the ACTN3 gene are prominent in power and endurance, respectively **(Kim *et al*., 2015).**

Stem cells differ from other kinds of cells in the body. All stem cells regardless of their source have three general properties: stem cells are unspecialized. However, unspecialized stem cell can give rise to specialized cells, including heart muscle cells, blood cells or nerve cells. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures and cannot work with its neighbors to pump blood through the body (like a heart muscle cell). It cannot carry molecules of oxygen through blood stream (like a red blood cell) and it cannot fire electrochemical signals to other cells that allow the body to move or speak (like a nerve cell) **(Dubie *et al*., 2014).** An acute bout of maximal exercise in adult athletes can double circulating CD^{34+} cells, increase white blood cell counts, and increase circulating burst-forming unit erythrocyte (BFU-E) and colony-forming unit granulocyte erythrocyte monocyte megakaryocyte (CFUGEMM) progenitors **(Morici *et al.*, 2005).**

The study aimed to determine the ACE and ACTININ 3 gene expression in trained and untrained Arabian horses and to show the acute response of exercise on gene expression. Moreover, our study aimed to use total RNA as a tool for fitness assessment. The study also tried to ascertain the relationship between hematopoietic stem cells (CD^{34+}) and exercise.

2- Materials and Methods:

2.1 Animals and Experimental Designs:

A total of 6 male Arabian horses were chosen from equestrian clubs in Cairo Governorate. Horses were divided into 2 groups of 3 untrained and 3 regularly trained animals. All horses were housed in individual boxes. Horses were traditionally fed with hay and a mix of cereals (oats and barley) provided three times a day (08:00, 12:00 and 20:00) and received water ad libitum. Untrained horses were kept inside their boxes and non of them were subjected to physical exercise. The groups of trained horses underwent training 4 days a week. The training session was performed in the morning from 7- 9 am. **The training include**: 100m…walking, 100-200m …trotting, 200-300m …narrow canter, 400-600m …wide canter, 100-200m …galloping.

2.1.1 Anthropometric measurements:

Body measurements (withers height, body length, heart girth and body weight) were measured. Withers height is the distance from the highest point of the processus spinali of the second and the sixth thoracic vertebra to the floor. Withers height was measured with measuring stick. Heart girth

represented by circumference of chest and measured by using plastic measuring tape. Body length was represented by the distance from the most cranial point of the sternum to the most caudal point of the pin bone and measured by measuring stick. Body weight according to the formula:

Weight (kg) = girth^{2}Xlength (both in cm)/ 11400

Table (1): represent Anthropometric measurements:

parameter	Length(Cm)	Girth(Cm)	Height(Cm)	Weight(Kg)
Untrained	162.33 ± 0.33	164.33 ± 0.33	151.67 ± 0.33	358.00 ± 0.58
Trained (Arabian)	154.00 ± 2.31	172.33 ± 1.45	155.00 ± 0.00	372.00 ± 2.89

2.1.2 Physical measurements:

Clinical studies of horses were performed before and after exercise, these clinical studies included the measurement of rectal temperature (T) and the assessment of heart rate (HR). Body temperature recorded by thermometer and heart rate recorded by auscultation.

2.2 Blood Samples:

Peripheral blood samples were obtained by jugular venipuncture from trained and non trained horses at rest time and immediately following the exercise in trained Arabian horses. Samples were aspirated into 10 ml syringes and immediately transferred into sterile K3 EDTA tubes and kept immediately in ice boxes, transferred immediately to biochemistry department, Kasr Al.Ainy, Faculty of Medicine, Cairo University for haematological testes, counting (CD^{34+}) and molecular biology tests (Total RNA measuring, ACE genotyping and Real time PCR for ACE and ACTININ 3 genes).

2.3 Molecular Analysis:

2.3.1 Measurement of total RNA

2.3.1.1 Isolation of PBMN cells:

Isolation of mononuclear cells from whole blood was performed through use of Ficoll-Paque Premium **(Biochrom, Berlin, Germany).**

2.3.1.2 Total RNA Extraction:

Total RNA was extracted from cell pellet using **SV Total RNA Isolation System kit, Promega, MadisonVVI, USA (Cat. # Z3100).** It was designed for isolation of total intranuclear RNA from fresh, whole blood treated anticoagulated with EDTA.

2.3.1.3 Quantitation and Storage of Total RNA

To determine 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

2.3.2 ACE genotyping

2.3.2.1 DNA extraction:

DNA extracted from whole blood samples using the kit supplied by Qiagen.

2.3.2.2 Polymerase Chain Reaction:

PCR amplification of the polymorphic region of ACE gene containing either the insertion (I) or deletion (D) fragment was performed. PCR was performed in a final volume of 50 µl, using the components of the mix produced by **Bio Basic INC** as follows (5 µl 10 x PCR buffer, 1 µl the 2 primers (50 Pmol for each), 1 µl 10 mM dNTPs, 1 µl Taq polymerase, 37 µl DEPC water) in a 45 ul total reaction volume. Only one pair of primers was used to determine ACE genotype.

Table (2): Sequence of ACE primers: (Rigat *et al.*, 1992).

5'- CTGGAGACCACTCCCATCCTTTCT -3'	**Forward primer**
5'- GATGTGGCCATCACATTCGTCAGAT -3'	**Reverse primer**

The ACE genotype, yielding amplification products of approximately 490 bp (for I allele) and 190 bp (for D allele). The tube was inserted in the thermal cycler and the thermal profile was adjusted to give (for each cycle): Denaturation at 95°C for 1 minute, Annealing at 60°C for 1 minute → for 35 cycles, Elongation at 72°C for 2 minutes, With an additional 10-minute incubation at 72°C after completion of the last cycle. Amplification products were visualized by using 1.5% agarose gels stained with ethidium bromide.

2.3.3 ACE and ACTININ 3 Real-time Polymerase Chain Reaction

2.3.3.1 Total RNA Extraction

Total RNA was extracted from cell pellet using **SV Total RNA Isolation System kit, Promega, MadisonVVI, USA (Cat. # Z3100).**

2.3.3.2 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.

2.3.3.3 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 different exons of each gene to avoid amplifying genomic DNA. The sequences of the primers and probes used in this study are listed in Table 3.

Table (3): Sequence of the primers used for real-time PCR

Primer sequence	Reference
Forward 5'- CTGGAGACCACTCCCATCCTTTCT -3' Reverse 5'- GATGTGGCCATCACATTCGTCAGAT -3'	ACE **Rigat *et al*., 1992)**
Forward :5`- CGGCGAGTATATGGAACAGG -3` Reverse: 5`- GTGAGTTGCACCAGGCAGT -3`	Actinin 3 **McGivney *et al.*, 2010)**
Forward 5'- CAAGGCTGTGGGCAAGGT -3' Reverse 5'- GGAAGGCCATGCCAGTGA -3'	GAPDH **Jiang *et al*., 2014)**

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 assessed using Applied Biosystem soft ware.

2.4 Stem cells counting:

Separation procedures were usually performed within 2-3 hours from collection.

2.4.1 Preparation of peripheral blood cells:

Anticoagulated blood was diluted 1:4 with PBS containing 2mM EDTA (Gibco- Invitrogen, Grand Island, NY) and 35ml of the diluted sample was carefully layered on 15 ml ficoll-Paque (Gibco-Invitrogen, Grand Island, NY), then they were centrifuged for 35 min at 400xg rpm. The upper layer was aspirated leaving the mononuclear cells (MNCs) layer undisturbed at the interphase. The interphase layer (MNCs layer) was carefully aspirated and washed twice in PBS containing 2mM EDTA and centrifuged for 10 minutes at 200 xg rpm at 20° c. The cell pellet was resuspended in a final volume of 300 µl of buffer.

2.4.2 Magnetic labeling of CD 34+ cells using MACS (Magnetic cell sorting) Kit (Mini MACS, Miltenyi Biotec, Bergisch Gladbach, Germany).

2.4.2.1 Magnetic labeling of cells in suspention:

100 µl of FcR blocking reagent was added to the cell suspension to initiate unspecific or Fc-receptor binding of CD^{34+} multisort microbeads to non- target cells. Cells were labeled by adding 100µl CD^{34+} multisort microbeads per 10^8 total cells and were incubated for 30 min in the refrigerator at 6° c- 12° c. After incubation, cells were washed carefully and resuspended in 300µl buffer.

2.4.2.2 Magnetic separation of CD34+ cells using MACS:

A positive selection column was placed in the Mini MACS separator and was washed with appropriate amount of buffer. Cell suspension was applied onto the column and negative cells were allowed to pass through. The column was removed from the separator, appropriate amount of the buffer was added and CD^{34+} cells collected by flushing out the column using the plunger supplied with the column.

2.4.3 Manual cell counting

Prepare a cell suspension in a balanced salt solution (eg. Hanks' Balanced Salts (HBSS) Product No.H 2513). Transfer 0.5 ml of 0.4 % Trypan Blue solution to a test tube. Add 0.3 ml of HBSS and 0.2 ml of the cell suspension (dilution factor=5) and mix thoroughly. Allow to stand for 5 to 15 minutes. With the cover slip in place, use a Pasteur pipette to transfer a small amount of Trypan Blue- cell suspension mixture to both chambers of the hemocytometer.

Cells per ml= the average count per square X dilution factor X 10^4

2.5 Statistical Analysis:

Data were analyzed with the Statistical Package for the Social Sciences (SPSS), version 21.0 and presented as mean ± standard error of means (S.E.M). Independent comparison t-test was used to compare the horse blood samples of trained and untrained horses. Paired comparison t-test was used to test the horse blood samples taken before and after exercise for any significant difference. Differences between groups were considered to be significant at P<.05.

3- Results and discussion:

3.1 Physical parameters

From table (4),the data revealed a significant decrease in **heart rate** in trained horses (**42.67 ± 1.33 beat/min**) as compared to untrained horses (**48.00 ± 0.00 beat/min**) ***(p<0.05)***. Our results are in agreement with **McKeever, (2003**) who reported that the increase in heart rate in the poor performance horses could be as a result of high core body temperature; this occurs

during the race, compromising cardiovascular function and heat loss mechanisms. The well-trained or physically well-fit aerobically individuals present a lower resting-HR, suggestive of higher parasympathetic activity (**Aubert *et al.*, 2001)** or lower sympathetic activity **(Chacon-Mikahil *et al.*, 1998)**. Exercise-induced bradycardia can also be due to intrinsic adaptation of the sinus node **(Catai *et al.*, 2002).** There was a highly significant increase in **heart rate** from (**42.67± 1.33 Beat/min**) before exercise to (**118.67±1.33 Beat/min**) after exercise as showed in table (5). Individual horses had a much higher rise in heart rate at entrance which can be attributed to the individual psychogenic response to stress **(Kastner *et al.*, 1999).** In the initial seconds of the exercise, HR increases due to inhibition of vagal activity, which not only increases atria contractility, but also conduction velocity of the ventricle depolarization wave from AV node **(Clausen, 1977)**. After this initial stage, as one goes on exercising HR increases again, due to adrenergic over stimulation on sinus node, or due to increase of serum norepinephrine, or atrial mechanics distention and therefore, sinus node distention due to a higher venous return, and the increase in body's temperature and blood's acidity **(Araujo, 1986).** In addition, long-term adaptive responses include hypertrophy of the cardiac muscle fibers (i.e., increases in the size of each fiber). This hypertrophy increases the muscle mass of the ventricles, permitting greater force to be exerted with each beat of the heart **(Wilmore and Costill, 1994).**

From table (4), the data revealed a non significant change in **temperature** between trained (**37.33 ± 0.09 °c**) and untrained horses (**37.10 ± 0.06 °c**).There was a highly significant increase in **temperature** from (**37.33±0.09 °c**) before exercise to (**39.07± 0.29 °c**) post exercise ***(p<0.01)*****table (5).** On agreement with **Piccione *et al.* (2011**)who claimed that, in horses, there is no correlation between rectal temperature and activity level and confirmed that rectal temperature maintained a similar circadian pattern regardless of exercise, indicating that the endogenous nature of its rhythm was not influenced by external stimuli such as physical exercise**. Janicki *et al.* (2013)** reported that the analysis of the results registered immediately after the exercise showed a statistically significant increase in the T (rectal temperature).

An increase in the internal temperature of the body induces a variety of responses, which prevent its further growth. The most important of these is increased heart rate and breathing, as a result of arousal centres of the circulatory and respiratory systems **(Janicki *et al.*, 2012).**

***Table (4)*: Mean values (±SD) and T test of physical parameters between trained and untrained horses:**

Parameter	Trained	Untrained	T-TEST
	Mean±SD	Mean±SD	
Heart Rate (Beat/min)	42.67 ± 1.33	48.00 ± 0.00	-4.000*
Temperature (°c)	37.33 ± 0.09	37.10 ± 0.06	2.214 ns

Mean values within a row with different letter superscripts differ significantly, *p < 0.05, ns: non significant

***Table (5):*Mean values (±SD) and paired comparison T test of physical parameters of Arabian horses before and after exercise**:

Parameter	Before	After	T-TEST
	Mean±SD	Mean±SD	
Heart Rate (Beat/min)	1.33± 42.67	1.33 ±118.67	-32.909**
Temperature (°c)	0.09± 37.33	0.29±39.07	-7.429**

Mean values within a row with different letter superscripts differ significantly, **p < 0.01

3.2 ACE Genotyping and gene expression:

From figure (1): There was no significant change in ACE genotype between trained and untrained Arabian horses, But the data revealed a high frequency of **D allele** among subjects. From ***table (6)***, There was a highly significant increase in **ACE** gene expression in trained horses (**3.35 ± 0.49**) as compared to untrained horses (**1.00 ± 0.00**) ***(p<0.01)***. **ACE** gene expression showed a significant increase between the rest (**3.35± 0.49**) and the post exercise period (**9.25 ± 0.61**) ***(p<0.05)*** as showed in ***table (7)***.

No association was found between sprint or vertical jump performance and genotype in the current study **(Gavin and Williams, 2010).** It might be inferred that ACE D allele positively contributes to an elite power athlete phenotype through its involvement in restricting local blood flow in skeletal muscles and therefore enhancing short-term exercise performance **(Jean-St-Michel *et al.*, 2011).** The suggestion of a

possible advantageous effect of the D allele in power sports is supported by the presence of an extraordinarily high frequency of the D allele in sprinters compared with controls and elite endurance runners **(Myerson *et al*., 1999).** Another study revealed an association of the D allele with exercise-induced cardiac hypertrophy in athletic subjects **(Fatini *et al*., 2000).**

Moreover, Our results agree with **Scott *et al.* (2005**) who reported that II and ID genotypes did not differ significantly in ACE activity, while DD genotype had a significantly higher ACE activity than both II and ID genotypes, in addition, In Thoroughbreds, ACE activity can be associated with successful racing, with horses displaying low ACE activity competing successfully at longer distances, while horses with higher ACE compete better at shorter distances **(Costa *et al.* 2009).** Furthermore, The athletes who present the ACE DD genotype, and therefore higher ACE activity in blood, demonstrate better results in sports which require speed, such as sprint running **(De Mello Costa and Slocombem, 2012).** The data support the findings of **Rush and Aultman, (2008)** who reported that in long-term training, increased ACE activity in the blood could be due to a modified genetic expression in response to the stimulus of exercise training**.** The decrease in ACE found with exercise training probably allowed a higher availability of bradykinin, which may have assisted in the process of vasodilation found in the data **(Alves *et al*., 2013).**

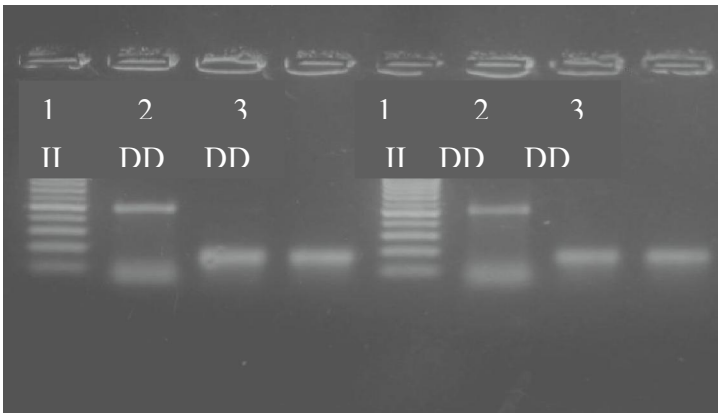

Figure (1): Determination of ACE I/D alleles by conventional PCR. (Separated on a 2% ethidium bromide-stained agarose gel under UV light). Amplification products of approximately 490 bp (for I allele) and 190 bp (for D allele).

3.3 Total RNA, and ACTININ 3 gene expression

The present work revealed a highly significant increase in **total RNA** in trained horses (**1430.00 ± 5.77 µg/ml**) than non trained horses (**510.00 ± 28.87 µg/ml**) ***(p<0.01)*** as showed in ***table (6***). The data showed in ***table (7)*** revealed a non significant change in **total RNA** before exercise (**1430.0 ± 5.77 µg /ml**) and after exercise (**1470.0 ± 5.77 µg /ml**). These findings are in agreement with **Capomaccio *et al.* (2011)** who reported that repeated bouts of exercise can lead to new basal levels of gene expression in resting tissues and horses seem to develop strong adaptation mechanisms capable of maintaining an anti-inflammatory body environment at rest.

Several studies performed on humans and horses have shown that changes in the expression levels of a wide range of mRNA transcripts appear to play a major role in the recovery of tissues following exercise with the expression levels of most genes returning to baseline within 24 h **(Capomaccio *et al.*, 2010).** Training augments the functional capacity of skeletal muscle, in part by altering the amount of proteins essential for contraction and energy metabolism **(Leisson *et al.*, 2008).**

The non significant change in total RNA before and after exercise in Arabian horses might be due to high pre exercise levels. The genes exhibiting the highest levels of expression at rest were more able to respond to the homeostatic changes induced by the race and further production of mRNA transcripts was unnecessary **(Cappelli *et al*., 2013).**The decrease in mRNA is also slow because they are rather stable (have a long half-life). As a result, the mRNA concentration does not return to baseline within 24 h, and the next training session takes place in the presence of a slightly elevate concentration compared to the day before **(Mougios, 2006).**

Moreover, a highly significant increase in **ACTININ 3** gene expression in trained horses (**4.40 ± 0.10**) than non trained horses (**1.00 ± 0.00**) ***(p<0.01)***. **ACTININ 3** gene expression showed a significant increase between the rest (**4.40 ± 0.10**) and the post

exercise period (**9.07± 0.73**) ***(p<0.05)*** as showed in ***table (7)***. Given the localization of α-actinin-3 to fast skeletal muscle fibers, we hypothesized that its absence would reduce performance in sprint/power events and would therefore be less frequent in elite sprint/power athletes **(North, 2008). Yang *et al.* (2003)** genotyped elite Caucasian athletes from various sports and demonstrated that both male and female elite sprint athletes had significantly higher frequencies of the 577R allele than the controls, suggesting that the presence of α-actinin-3 has a beneficial effect on the function of skeletal muscle in generating forceful contractions at high velocity**.** Moreover, subjects without ACTN3 gene expression seem better able to adapt to a stressful condition **(Clarkson *et al.*, 2005)**, but they are unable to develop power to the same extent as persons with the 577R allele**.** The mechanism of muscle hypertrophy in response to overloading begins with a rise in cytosolic Ca^{+2}. Ca^{+2} binds to calmodulin. Upon bindind ca^{+2}, calmodulin can associate with several proteins and modify their biological activity. Two such proteins are calmodulin-dependent protein kinase and protein phosphatase 2B, the latter of which is commonly known as calcineurin. These enzymes phosphorylate and dephosphorylate respectively, key transcription factors controlling certain genes involved in the hypertrophic response **(Mougios, 2006)**.

***Table (6)*: Mean values (±SD) and T test of total RNA, ACE and ACTININ 3 gene expression between trained and untrained horses**

Parameter	Trained	Untrained	T-TEST
	Mean±SD	Mean±SD	
Total RNA(µg/ml)	1430.00 ± 5.77	510.00 ± 28.87	31.251**
ACE	3.35 ± 0.49	1.00 ± 0.00	4.789**
Actinin 3	4.40 ± 0.10	1.00 ± 0.00	34.000**

Mean values within a row with different letter superscripts differ significantly, **p < 0.01

***Table (7):* Mean values (±SD) and paired comparison T test of Total RNA, ACE and ACTININ 3 gene expression of Arabian horses before and after exercise**:

Parameter	Before	After	T-TEST
	Mean±SD	Mean±SD	
Total RNA(µg /ml)	5.77 ± 1430.0	5.77 ± 1470.0	-4.00 ns
ACE	0.49 ± 3.35	0.61 ± 9.25	5.378*-
Actinin 3	0.10± 4.40	0.73 ± 9.07	-6.614*

mean values within a row with different letter superscripts differ significantly, *p < 0.05, ns: non significant

3.4 Stem cells

There was markedly increase in **stem cells** numbers in trained horses with mean concentrations of (**7746.70 ± 430.52 cell/ml**) as compared to untrained horses with mean concentrations of (**3451.00 ± 227.85 cell/ml**) ***(p<0.01)***. Markedly increase of **stem cells** numbers was observed. The stem cells numbers raised from (**7656.4 ± 522.20 cell/ml**) at rest to (**11126.00 ± 843.86 cell/ml**) immediately after exercise ***(p<0.01)*** **(table 9)**. **Amani and Mohamed (2011)** proved that a two month training program can improve values of physical variables and time together with increased stem cells among young runners. **Shalaby *et al.* (2012)** reported that as for the adaptive response to anaerobic exercise, blood cellular components of RBCs, WBCs, HCT and haemogbin numbers and contents increased together with increase CD^{34+}compared to aerobic one and control CD^{34+}**.** Our data support the findings of **Common *et al.* (2013)** who reported that the baseline levels of primitive stem cells in adult equine blood were shown to increase in the presence of moderate exercise**.** Stress, both physiological and induced, including exercise, clinical G-CSF stimulations, bleeding, organ injury, inflammation is accompanied by enhanced release of catecholamines and upregulation ofb-AdR expression on HSPC. These are associated with a reduction in SDF-1 levels in the BM and increase in the peripheral blood, as well as increased CXCR4 expression in the BM. In addition, these stress conditions trigger expansion and activation of osteoclasts, and the release of various proteolytic enzymes enabling the robust mobilization of HSPC from the BM to the bloodstream to participate in host defense and organ repair **(Spiegel *et al.*, 2008).**

Table (8): **Mean values (±SD) and T test of stem cells numbers between trained and untrained horses:**

Parameter	Trained	Untrained	T-TEST
	Mean±SD	Mean±SD	
Stem Cells (cell/ml)	7746.70 ± 430.52	3451.00 ± 227.85	8.819**

Table (9): **Mean values (±SD) and paired comparison T test of stem cells number of Arabian horses before and after exercise:**

Parameter	Before	After	T-TEST
	Mean±SD	Mean±SD	
Stem Cells (cell/ml)	522.20 ± 7656.4	843.86± 11126.00	-5.045**

4. Conclusion:

In conclusion, the data in this paper support the fundamental role of performance genes especially ACE and ACTININ 3 genes in selection of racing horses. In addition, the paper proved the fact that frequencies of stem cells can be increased by exercise suggests the importance of stem cells in selection of elite horses. Interestingly, the total RNA alone can be used a good indicator for individual variations between racing horses. The integrative action of both stem cells and performance genes in selection of racing horses is due to the fact that stem cells provide muscle fiber and the genes expressions provide the bulk of the muscle due to protein synthesis.

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