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Circulating Hematopoietic Stem Cell and Some Physiological Parameters in Different Training Programs

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Abstract: Exercise is one of the most powerful non pharmacological strategies, which is able to affect nearly all cells and organs in the body. Changes in the behavior of adult stem cells have been shown to occur in response to exercise training. The aim of this study is to reveal the role of aerobic and anaerobic training programs on CD^{34+} stem cells and some physiological parameters .20 healthy male athletes aged (18-24 yrs) were recruited for this study. Healthy low active males and BMI matched participants (n=10) aged (20-22 yrs) were recruited as controls .Aerobic and anaerobic training programs for 12 weeks were used. Vo_{2max}, pulse rate estimation using strand Rhyming protocol. RBCs,WBCs,Hb and hematocrit were estimated using coulter counter, Lactate by accusport,CD³⁴⁺ stem cells by flow cytometer. Results revealed: VO_{2 max} was increased in case of aerobic training program compared to anaerobic one .Lactate concentration was decreased in case of aerobic training programs compared to anaerobic one. RBCs, Wbcs, Hb and hematocrit were increased in anaerobic than aerobic training programs CD³⁴⁺ stem cells were increased in case of anaerobic training program than aerobic one and control. It is concluded that training programs provoke better adaptation

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Key words: Aerobic and anaerobic training programs, CD34⁺ stem cells, physiological parameters.

1. Introduction

Exercise is one of the most powerful nonpharmacological strategies, which is able to affect nearly all cells and organs in the body. In this contest, a new research avenue focusing on the action of exercise on adult stem cells has emerged during the last decade. Changes in the behavior of adult stem cells from different regions including skeletal muscle and the cardiovascular system have been shown to occur in response to exercise training.

Through its action on adult stem cells, exercise may act on the regenerative potential of tissues by altering the ability to generate new stem cells and differentiated cells that are able to carry out tissue specific functions (Kado and Thonell, 2000). Strength and power are important aspects of fitness, sport and everyday activity. However, much debate remains as to how these qualities, should be assessed. Much of the debate originates from the definition of strength and power and the different terminology used across laboratories. Sale (1991) defined strength as the force exerted under a given set of conditions during a maximal voluntary contraction (MVC). Sale continued to define power as the rate at which mechanical work is performed under a specified set of conditions, or the product of force and velocity. Both definitions imply that strength and power are defined by conditions such as velocity, contraction type, and posture and movement pattern specificity. That is, strength for one task may not imply strength for another. An associated problem with this is that strength and power are quite often measured in contexts dissimilar to the environment in which functional strength and power are needed (Fatourous et al., 2000).

There are several training methods which are used to enhancement of strength and power of these methods is the complex circuit exercises together with the well known aerobic and anaerobic methods which are used in this thesis.

Guyton and Hall (2006), reported the effect of athletic training on muscles, they stated that muscles that function under no load, even if they are exercised for hours on end, increase little in strength. At the other extreme, muscles that contract at more than 50% maximal force of contraction will develop strength rapidly even if the contraction are performed only a few times each day. They also added that during muscle contraction blood flow increase about 13 fold but also the flow decrease during each muscle contraction, this decrease in flow is due to the compression of intramuscular blood vessel, but the blood flow to muscle increases during contraction.

Thomas Hawke (2005) stated that although endurance training is associated with high repetition low resistance exercise, signification muscle damage can occur if the duration or mode of exercise is extreme, for example, both marathon running and downhill running can lead to significant muscle fiber damage. In contrast to endurance training, resistant exercise training is associated with high intensity. Low repetition work loading to increases in muscular strength, power and oxidative capacity, with little change in aerobic capacity. The workloads placed on skeletal muscle during resistance training are at or near maximal capacity, and as such produce significant perturbations to other skeletal muscle fibers and the associated extracellular matrix.

In a recent study, Burd *et al.* (2010), investigate the impact of two distinctly different exercise volumes on anabolic signaling myogenic gene expression, and rates of muscle protein synthesis (Mix, Myo, Sarg), specifically, they utilized a unilateral model in which subjects performed exercise at 90% IRM until failure (90 FAIL), 30% IRM in which the amount of external work was matched to 90 FAIL (30 WM), or 30% IRM to failure (30 FAIL). They reached the conclusion that low-load high volume resistance exercise is more effective in inducing acute muscle anabolism than high load low volume or work matched resistance exercises modes.

As for training induced adaptations, exercise induced neutrophilia was shown to become progressively blunted with training (Suzuki *et al.*, 1999), but no study ever tested whether circulating HPC counts may differ between trained and sedentary subjects. Circulating immature cells are likely involved in angiogenesis (Reyes *et al.*, 2002) and repair processes (Springer *et al.*, 2001) both mechanisms being possibly associated with strenuous exercise and progressive training. Given the large use of exercise based rehabilitation programs in several diseases, knowledge of the physiological effects of training on HPCs might be of potential clinical use.

Identification of EPCs on the cell surface expressions of various protein markers. There is no straight forward definition of an EPC marker because these cells seem to be a heterogeneous group associated with different cell surface antigen expression profiles. The most commonly described molecules that serve as biomarkers for recognition of an EPC population include CD^{34} +, CD133, and VEGFR2. The pioneer study of Asahara *et al.* (1999) recognized EPCs as CD^{34+} mononuclear cells (MNCs). Hematopoeietic stem cells that serve as a source of EPCs express CD^{34+} , however this marker is also present on the surface of mature endothelial cells (Fina *et al.*, 1990).

Human CD133 antigen is a membrane glycoprotein whose expression is related to hematopoeitic stem cell differentiation into EPCs (Urbich and Dimmeler, 2004). The third marker proposed for EPC identification is VEGFR2, a protein predominantly expressed on the endothelial cell surface. Urbich and Dimmeler, (2004) and Birn *et al.* (2005) claim that EPCs are positive for CD^{34+} , CD133 and VEGFR2 markers.

 CD^{34+} cells are multipotentprogenitors that can engraft in several tissues (Krause *et al.*, 2001), circulating CD^{34+} cells can be used to indirectly estimate hematopoiesis based on CD38, human leukocyte antigen (HLA) Dr, and CD33 markers..

Patrick and Stephane (2003) found that CD^{34+} stem cell from elite triathletes to be significantly lower than in healthy sedentary subjects. They stated that the low CD^{34+} counts and neutopenia and low lymphocyte counts could contribute to the increased upper respiratory tract infections observed in these sportsmen. They hypothesized three explanations (1) Aerobic training could induce deleterious effect on BM by inhibition of central CD^{34+} SC growth (2) intense training could depress the mobilization of CD^{34+} SC. (3) due to aetology of the damage/ repair process. They conclude that CD^{34+} SC quantification in elite sports men should be helpful for both basic science researches and sport clinicians.

The aim of this study is to reveal the role of aerobic and anaerobic training programs on CD³⁴⁺ stem cells and some physiological parameters.

2. Material and Methods Participants:

Twenty healthy male athletes aged (18-24 yrs) with a training history of (4-9yrs) were recruited for this study. Athletes have to participate in low to intense exercise greater than 3 days/week. Healthy low active male and BMI matched participants (n=10) aged (20-22yrs) were recruited as controls. Control subjects could not be participating in or have a recent history of low to intense regular exercise. Participants were screened and asked to fill out healthy history and physical activity history questionnaires.

All participants were non smokers, non diabetic and free of cardiovascular, lung, liver disease. Participants did not take any medications that affect EPCs number or function. These include statins, angiotensin ll receptor antagonists, ACE inhibitors; peroxi some proliferators activated receptor (PPAR α) agonist and EPO.

Testing procedures

Written informed consent was obtained for all participants and the study was approved by the University of Suez Canal institutional reviews board. All participants engaged in a preliminary screening visit to evaluate resting blood pressure and fasting blood chemistry profile, and to rule out the presence of cardiovascular disease and to assess and obtain samples of blood for analyses and BMI testing.

They were given a weight data log and instructed to weight themselves in the morning and evening and record their weights in the log. All participants refrained from caffeine and any medications or vitamins 48 hours prior to the test. Participants were instructed to record their intake of foods for the three days before test on a log supplied to them.

Athletes were divided into two groups, one group was subjected to aerobic training program and the other group was subjected to anaerobic training program. The training program lasted for 12 weeks for each group each protocol was composed of warming up for several minutes, then the training cases which was ended by cooling down procedure for another minutes.Vo_{2 max} value is obtained using Astrand Rhyming nomogram RBCs, WBCs Hb and hematocrit value were estimated using coulter counter.

The human erythrocyte is the mature unit of the red blood corpuscle; it is circular, elastic nonnucleated, biconcave disc, whose primary function is the transport of hemoglobin. Hemoglobin is a protein of 200 to 300 million nearly spherical molecules in each red blood cell, having a molecular weight of 64.458 based on the chemical structures of its alpha and beta chains. Hematocrit (the packed cell volume) is the percentage of the total volume of whole blood that is occupied by packed red blood cell when a known volume of whole blood is centrifuged at a constant speed for a constant period of time. White blood corpuscle (leukocyte) includes all white cells of the blood, lymphocyte, monocyte neutrophil and basophil and eosinophil(Guyton and Hall, 2006).All blood cells were counted using coulter counter which is easy to read numerical presentation. Lactate analysis was performed by using accusport after the training programs at rest compared to control at rest.

Circulating progenitor cell number:

 CD^{34+} (HPc, hematopoietic progenitor cell number was determined by flow cytometry for this assay 0.5 ml of blood was collected into an EDTAcoated tube. Mononuclear cells were separated via density centrifugation. Cells were washed and counted with a hemocytometer. Mononuclear cell were immunostained with monoclonal anti-bodies against human CD³⁴⁺ for each group of analyses, one set of control tubes for machine calibration was generated. Flow cytometry was performed in a special laboratory. The forward side scatter plot was used to identify lymphocyte gate. 100.000 events per sample were acquired. Total cell count was averaged. The following principle, clinical applications precautions and methodology in the following:

IOTestCD³⁴⁺PE:

Use this fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the CD³⁴⁺ antigen present in human biological samples using flow cytometry.

Statistical Analysis

Student's t tests were used to test for differences between athletes and control groups and between aerobic and anaerobic groups where data were found to not meet the assumption of normality, the nonparametric Mann Whitney u test (Wilcoxon rank sum test) was used to compare difference between groups. In these cases, for descriptive data the median (Lowest value-highest value) are displayed. Difference between groups was testing using a measure of analysis of variance (ANOVA). For parameters with non normal distributions non parametric Spearman correlation coefficients were used.F test was used to test 3 groups. An α level of 0.05 was used to indicate statistical significance.

Aerobic Training Program After*Dr. Phil Esten(2010)*

Physiologically speaking, the 3000, and 5000 meter events pull up to 80 percent of their performance energy from the oxidative energy system. Most of us know this system as the aerobic energy system. Therefore, 70 to 80 percent of training should be actual distance or aerobic running, which activates the oxidative energy system.

Anaerobic Training Program After Tom Green (2003)

Sprinting is a difficult combination of aggression, relaxation, technique and efficiency. The 100 meters is sometimes labeled as the easiest most complicated event in sport! And contrasting bodybuilding, gaining too much size can become a negative. Generally speaking world-class sprinters are not that large, anywhere from 155-180lbs. In fact, what's interesting is that some sprinters do not lift weights at all! But for those of us who aren't as genetically gifted, the ultimate goal is having incredible strength-to-weight ratios, lean body mass and a well-developed CNS (central nervous system) for fast reaction and the ability to explode on command.

3. Results

Subjects characteristics:

Twenty athletes and 10 low active control males participated in the study. Groups were matched for age, weight and height (Table 1). Also for BMI, nonsignificant changes in basic characteristics, to compare athletes and control males.

Pulse rate and VO_{2 max} showed significant changes (Table 1), as expected athletes had a lower pulse rate compared to control. Physical activity questionnaire data revealed that athletes exercised an average of 5 ± 0.5 days a week for 5 ± 0.2 years.

Control group participants were not engaging in regular exercise, nor did they have a recent history of physical activity.

Twenty Athletes agreed to participate in 12 weeks of training sessions of aerobic and anaerobic exercises.

Table (1)	: Basic	characteristics
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Variable	Athletes	(N=20)		Control (N=10)			Sig.	
Age (yr.)	21.6	±	1.83	20.6	±	0.89	NS	
Height (cm)	179	±	2.78	178.8	±	1.92	NS	
Weight (kg)	75	±	3.16	74	±	1.5	NS	
BMI	22	±	1.4	23	±	2.2	NS	
Pulse rate (count/m)	68	±	2.3	74	±	2.1	S	
$VO_{2max}(ml/kg)$	52	±	1.8	36	±	1.7	S	
Lactate (mmol/L)	1.1	±	0.02	1.2	±	0.03	NS	

Values are means \pm SE *P*<0.05; BMI = body mass index

 Table (2): Haematopoietic stem cells for control, aerobic exercise training and anaerobic training courses of exercise for 12 weeks in the resting stages and Lactate

Variable	Control		Aerobic training A					Anaerobic training		
CD ³⁴⁺ S cells	170.0	±	21.10	130	±	14.61	251.6	±	21,64	
Lactate (mmol/L)	1.2	±	0.3	0.8	±	0.1	0.9	±	0.2	

Table (2) reveals a significant changes after anaerobic training compared to aerobic and control in case of CD^{34+} SC(values are means ±SE *P*< 0.05).

 Table (3): Haematological values of RBCs, WBCs, HB and hematocrit (PCV) after aerobic and anaerobic training program (at rest) and control.

Variable	Contro	l		Aerobic	trainir	ıg	Anaerob	oic traini	ng	Sig
RBCs (million/mm3)	4.7	±	0.9	4.9	±	0.2	5.3	±	0.3	S
WBCs (thousands/ mm3)	4.8	\pm	0.7	6.1	\pm	0.4	6.6	\pm	0.5	S
Hb (g/dL)	12.8	\pm	0.8	14.2	±	0.5	15.4	±	0.4	S
Hematocrit (%)	42	±	3.2	44	±	1.1	46	±	1.2	S

Table (3) reveals a significant change between participant in aerobic program and anaerobic one in hematological value and control (P < 0.05).

Table (4): The variation in $VO_{2 max}$ or participants healthy sedentary and after aerobic and anaerobic training programs.

Participants	VO _{2max} (mL/kg/min)					
Healthy sedentary (mL/kg/m)	36	±	1.7			
Aerobic training program (mL/kg/m)	62	±	2.2			
Anaerobic training program	54	±	2.1			

The results are expressed as mean \pm SE (*P*<0.05).

Table (4) $VO_{2 max}$ (mL/kg/min) results indicated an increased value between the healthy sedentary participants and after aerobic and anaerobic training programs.

4. Discussion

Sport's training is done for improving sports performance. The sports performance as any other type of human performance is not the product of one single system or aspect of human personality. On the contrary, it is the product of the total personality of the sports person. The personality of a person has several dimensions of physical, physiological, social and psychic. Sports training, therefore, directly and indirectly aim at improving the personality and fitness of the sport man. The specify of training principle states that the nature of tissue adaptation after training is dependent on the specific type of training practiced (Nieman, 2003).

Tables (1,2) revealed lower values of lactate after aerobic and anaerobic training programs which means a better fitness. Lactate is the end product of the anaerobic carbohydrate breakdown. It is the metabolite displaying the most spectacular concentration changes in muscle and the blood with exercise. As a result, its measurement offers a wealth of information regarding the effect of exercise on metabolism. Lactate determine in whole blood rather than plasma or serum. (Mougios, 2006) He also added that when measuring lactate in the blood after short hard or maximal exercise it is necessary to remember that it takes some minutes to peak. Thus, a blood sample taken right after the end of exercise will not produce the peak value. To trace it, it is needed to perform several minutes before taking the samples.

Programming training based on intensities dictated by blood lactate concentration is superior to programming training based on heart frequencies

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because lactate relates directly to muscle metabolism and muscle adaptations. Thus, one could use lactate to determine training intensities at the beginning of a training program, monitor training through heart frequencies on a daily basis, and resort to lactate periodically every few weeks to fine tune intensities. Most investigators agree that intensities that hold below the blood lactate concentration below 4mmol/L are the most effective in improving aerobic endurance, cardiac function and the lipidemic profile (Greenhaff and Timmons, 1998; Mougios, 2006),

Barrett et al. (2010) in Ganong review of Medical physiology stated that blood consists of a protein rich fluid known as plasma, in which are suspended cellular elements: white blood cells, red blood cells and platelets. The normal total circulating blood volume is about 8% of the body weight (5600 ml in a 70 kg man). About 55% of this volume is plasma. They added that red cells, white cells and platelets are formed in the bone marrow, which is actually one of the largest organs in the body, approaching the size and weight of the liver. Hematopoietic stem cells (HSCS) are bone marrow cells that are capable of producing all types of blood cells. They differentiate into committed stem cells (Progenitors cells). The HSCS are derived from uncommitted, tot potent stem cells that can be stimulated to form any cell in the body, adults have a few of these, but they are more readily obtained from the blastocysts of Embryo.

Robergs and Roberts (1997) stated that the main functions of the cellular components of blood are the transport of oxygen and carbon dioxide, blood clotting, acid base buffering immune functions and tissue repair and destruction, and the function of plasma (liquid components) are blood clotting, circulating or cellular components and their contents, heat transfer and thermoregulation, water exchange and transport, circulation of hormones, acid base buffering, circulation of metabolites, nutrients and waste products.

Gillen et al. (1991), Burge et al. (1993 reported that acute effect of exercise on blood is to cause release of fluid from the vascular component, which decreases the volume of plasma and blood. This fluid loss from the plasma decreases plasma volume and cause hematocrit and plasma metabolite concentration to increase, which is termed hemoconcentration. In fact, a significant hemoconcentration occurs when a person moves from a supine to a vertical position. The added hemoconcentration of exercise is predominantly confined to the transition from rest to exercise. This is followed by a more response gradual hemoconcentration that occurs with increases in exercise intensity. And these changes are larger during the larger blood pressure associated with resistance exercise than during more prolonged dynamic exercise.

Spriet *et al.*(1986) added that prolonged exercise involving sweating increased fluid loss from the body,

and the degree of hemoconcentration can be measured by either directly measuring plasma volume or estimating relative changes in plasma volume from hemoglobin and hematocrit measurements. Also blood viscosity increases above what would be expected for hemoconcentration effects. In addition there is destruction of erythrocyte, termed hemolysis, which increases plasma hemoglobin concentration (Zierler *et al.*, 1992). This was in accordance with the increased cellular changes after training programs due to hemoconcentration (Table 3).

Endurance training increases the volume of blood the ventricle can hold and contributes to its maximum stroke, ventricular thickness is usually slightly increase. The blood cells, Rbs, Wbcs, platelets and Hematocrit and hemoglobin are slightly increase together with stem cells SC, CD^{34+} (Tables 2,3).

As for the adaptive response to anaerobic exercise, blood cellular components of RBCs, WBCs, HCT and haemogbin numbers and contents increased together with increase $CD^{34+}SC$ compared to aerobic one and control CD^{34+} (25,6±21,64) (130±14,61)and 170±21.10 (Tables 2.3), this was in accordance with the results of Bonsignore *et al.* (2002) Mobius – Winkler *et al.*, 2009 Bonsignore *et al.*, (2010), ,.

As for table(4), it indicated an increased Vo_{2max} value between sedentary , aerobic and anaerobic training programs participants. Also a significant change in aerobic compared to anaerobic training program which means a better cardiovascular adaption for the aerobic grop.

Amany and Mohamed (2011) reported the effect of concurrent training (endurance and resistance on CD^{34+}/CD^{45+} stem cells, VO_2 max, certain physical variables and record level of 1500 m. running, they came to the results that there was a significant increase between pre and post measures in accounting of CD^{34+}/CD^{45+} stem cells, power and strength, VO_2 maxand record level of 1500m running for the sake of concurrent training group they concluded that the concurrent training for two months can improve physical, and the record level together with increased stem cells among young runners.

Resistance exercise stimulates the synthesis of skeletal muscle proteins (West *et al.*, 2009), which is expressed as muscle hypertrophy .It has recently been established that, myofibrillar (My) protein synthesis is already maximally stimulated at 60% IRM, in the post absorptive state, with no further increase at higher load intensities (ie 75 - 90 % IRM) (Kumar *et al.*, 2008).

Additionally, performance of low load contraction (~ 20 IMR) with vascular occlusion is sufficient to induce an increase in mixed muscle (Mix) protein synthesis (Fujita *et al.*, 2007).

In 2010, Burd *et al.*, reported that low-load high volume resistance exercise is more effective in inducing acute muscle anabolism than high-load low

volume or work matched resistance exercise modes. Fifteen young men $(21\pm 1 \text{ years})$, performed 4 sets of unilateral leg extension exercise at different exercise loads and/or volumes. 90% of repitation maximum (IRM) until volitional failure (90 Fail) 30% IRM work matched to 90% fail (30 wM), or 30% IRM performed until volitional failure (30 FAIL).

Regular physical activity is associated with enhanced endothelial function which has been related to lower incidence of cardiovascular disease (Delp *et al.*, 1993; Delp 1995; Hambrecht *et al.*, 2003 and Haram *et al.*, 2006).

Bonsignore et al. (2002) suggested that increased HPCS reflect on adaptation response to recurrent, exercise-associated release of neutrophils and stress and inflammatory mediators, indicating modulation of bone marrow activity to habitual running. In 2004, Laufs et al., measured EPCS in mice and patients with stable CAD. Mice engaged in 3 weeks of voluntary wheel running and humans underwent a 4 week training program of bicycle ergometer endurance exercise (60- 80% preak Vo_{2max}), strength exercise, and walking. EPC number was significantly increased in the blood, bone marrow of mice after 7 days of exercise which persisted for the 28 days of the training program. In human the number increased $78 \pm$ 34% compared to before the 4 week training program. EPC apoptosis was found to decrease 41 ± 11 % after training. As for Steiner et al. (2005) who utilized a 12 week exercise program in patients with asymptomatic coronary artery disease (CAD). Results showed a 2.9 \pm 0.4 fold increase in circulating EPcs in the exercise group. This increase was correlated with an increase in flow mediated dilation and no synthesis.

In another training study, Sandri et al. (2005) analyzed the responses of circulating (CD^{34±}/KDR [±]) number and function in three patient groups, those with ischemic and training occurred for 4 weeks. Increases in CPC was increased in all three groups accompanied by an increase in the CXCR4, also VEGF levels were increased in the groups. They concluded that the ischemic exercise groups appeared to increase VEGF, which may have stimulated the increase in CPC numbers. Thijssen et al. (2006) reported no change in $CD^{34+}\!/\ KD^{\pm}$ cells in healthy young and older participants following 8 weeks of cycle exercise training for 20 minutes 3 time per weeks at 65% of heart rate reserve. Therefore, exercise training may not increase EPC number in healthy individuals. Vasankari et al. (1998), Hoetzer et al. (2007) reported that exercise may improve the number and function of EPCS while improving oxidative stress status.

Conclusion

It may be concluded that:

• Vo_{2 max} was increased in case of aerobic training program compared to anaerobic one and control indicating a better cardiovascular adaptivity.

- Lactate concentration was decreased in case of aerobic training programand anaerobic one compared to control meaning a better fitness.
- Hb, RBCs, WBCs and hematocrit value were increased after anaerobic training program compared to aerobic one due to stress.
- CD³⁴⁺ SC counts were increased in peripheral blood of anaerobic training program then aerobic one and control due to stress and indicating better adaptation.

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