Efficiency of Exercise Program on CD 34$^+$ Stem Cell, Blood components and Some Physical and Skill Variables

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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 program. The aim of this study is to reveal the role of exercise program on CD34$^+$ stem cells, some blood components, physical and skill levels for Third year with low levels in physical education. 10 healthy female athletes aged (19-21 yrs) were recruited for this study. Exercise program for 8 weeks (3 days / week) were used aerobic exercise steps, bar exercise and skills Training.They were estimated using Immunophenotyping CD34$^+$ stem cells, Complete blood count (CBC), Harvard step Test for Physical Assessment and Assessment of skills. Results: revealed to significant increases of HB, MCH, RDW, MCV, MCHC, Leucocytic count (WBCs), CD 34$^+$ %, CD34+ Count. It is concluded that exercise program affect all parameters positively.
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Key words: Exercise Program, CD34$^+$ stem cells, Blood components, Harvard step test for Physical Assessment, Aerobic exercise steps, bar exercise, Skills training.

1. Introduction

Witness the science every day progressed and a stunning advance in various fields and scientific research comes every day new. Stepping sports training and strides along with other bioscience for the service of humanity. Sports training help the changes of the body's physiological and biological components may be temporary in direct response to physical effort or permanently as a result of the continued attendance at training.

Stem cells are unspecialized cells that have two defining properties. the ability to differentiate into other cells and the ability to self-regenerate. (Asahara and Isner, 2004),(Lindblad, 2004).

The ability to differentiate is the potential to develop into other cell types. A totipotent stem cell (e.g. fertilized egg) can develop into all cell types including the embryonic membranes. Multipotent: a small number of stem cells can produce only certain types of cells. A pleuripotent stem cell can develop into cells from all three germinal layers (e.g cells from the inner cell mass). Other cells can be oligopotent, bipotent or unipotent depending on their ability to develop into few, two or one other cell type(s). (Priller, (2004), (Davila et al (2004).

Self-regeneration is the ability of stem cells to divide and produce more stem cells. During early development, the cell division is symmetrical i.e. each cell divides to gives rise to daughter cells each with the same potential. Later in development, the cell divides asymmetrically with one of the daughter cells produced also a stem cell and the other a more differentiated cell. (Barker, Widner, (2004), (Floss, Wurst, (2002).

CD34 $^+$ is expressed protein, and the first indicator marrow cells derived from blood and bone, and expression (CD34$^+$) was only shown the first of the characteristics of stem cells vessels (HSC), which is the main source for the production of red blood cells and white blood cells, platelets, which makes stem cells bloody gold standards for all types of stem cell cellular therapy, and although the biological functions of CD34$^+$ is largely unknown, and recent data suggest that CD34$^+$ involved in maintaining stem cells in an undifferentiated state outwardly, and master cells, and primary cells of hematopoietic, so the CD34$^+$ stem cells are needed for further studies, to identify their directed differentiation into more specialized types of blood cells.(Heshmat and Roshdy (2011).They also added that the enormous capacity of adult stem cells for growth and reform and re-generation of damaged cells and tissues in the body which is a bag repair or maintenance staff need only to activate and reform what is devastating.

Thus, the stem cells in turn depends on the socalled «old fetal» of the body. There are stem cells that generate the ability to make anything. Then there are the stem cells «College Ability», which can make more type of tissue, and then there are adult stem cells that proliferate to create a special texture to the body, such as the liver or bone marrow or skin.Etc... Thus, with each step toward adulthood, the successes

achieved by the stem cells are narrower, which means that lead to specialization. In adulthood, does not generate liver cells, but other liver cells, skin cells, generate another. However, the sign of recent research suggests that the amount of cells can be manipulated to return back and enable it to produce various tissues, such as conversion of bone cells to produce muscle tissue. There are stem cells in two forms: Embrionic stem cells, and adult stem cells. (Rehman et al, 2004), (Barrett et al, (2010). As for training induced adaptations, exercise induced neutrophilia was shown to become progressively blunted with training (Suzuki *et al.,* 1999).

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 Thornell, 2000).

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.

$CD34^+$ cells are multipotent progenitors that can engraft in several tissues (Krause *et al.,* 2001). 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, 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. 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.

Some studies also showed that the exercises improve the function and re-composition of the cardiac and circulatory system and musculature that activate and transfer of stem cells or blood stem cell collection.(Patrick, et al 2008)

Laura Bilek (2008) studied the relationship between physical exertion and stem cells for adults where proved increase the number of stem cells as a result of exertion physical linked better health for the elderly, and there is an inverse relationship between stem cells and age.

Since the stem cells are the key ingredient of red blood cells, platelets and white blood cells next to the somatic cells and components of the muscle fibers, and heart, nerve and fibers association. So we desperately need to their increase for humans in general and sports in particular, to improve the capacity and efficiency of vital organs as cardiovascular and nervous and muscular, which would bring human physical and mental efficiency and health. The researchers observed from their experience in the academic field that there are some students (with low levels physically and skilly) with an unwillingness to continue the performance and the effort. So the researchers developed an exercise program for physical and skill to increase and improve the efficiency of stem cell CD 34^+ and blood components to students of third year Physical Education College in attempt to upgrade their performance and improve their level of fitness and health.

The Aim of this study is to reveal:-

1. The role of exercise Program on $CD34^+$.
2. The role of exercise Program on some blood components.
3. The role of exercise Program on physical and skill levels.

Research hypothesis:

1- There are statistically significant differences on $CD34^+$ between pre and post program in favour of the post measurement.

2- There are statistically significant differences on some blood components between pre and post program in favour of the post measurement.

3- There are statistically significant differences on physical and skill levels between pre and post program in favour of the post measurement.

2. Materials and Methods

Participants:

10 healthy female athletes aged (19-21yrs) were recruited for this study, they were in the third grade in the faculty of physical education for girls in Elgezira. They have to participate in an exercise program for 8 weeks (3 days/ week). Exercise program includes three items 1) Warm up, 2) Main exercise composed of aerobic exercise steps,bar exercise, skills training 3) Cool down.

All participants were nonsmokers, non-diabetic and free of cardiovascular, lung, and liver disease.Participants did not take any medications that

could affect EPCs number or function. These included statins, angiotensin II receptor antagonists, ACE inhibitors; peroxisome proliferators activated receptor (PPARα) agonist and EPO.

Testing procedures:

Written informed consent was obtained for all participants. All participants refrained from caffeine, vitamins, and any medications 48 hours prior to test, which include blood withdrawal before and after exercise program.

Table (1) The basic characteristics were tabulated for $CD34^+$ counts and percent, blood components for all participants in (n=10)

S	Variable	Mean	SD.	Range		Sk
				small	big	
1	Hemoglobin gm/dl	12.37	0.88	11.40	14.10	0.616
2	Red cell count mil/cmm	4.433	0.429	4.02	5.48	18.41
3	MCH pg	28.07	2.649	21.00	30.70	-2.434
4	RDW	12.89	0.941	11.80	14.90	1.008
5	Hematocrit %	36.87	2.436	34.00	42.40	1.224
6	MCV fL	83.66	7.034	65.00	90.30	2.403
7	MCHC gm/dl	33.52	0.609	32.30	34.30	0.761
8	Total leucocytic count/ cmm	7.57	1.500	4.90	9.70	-0.461
9	Platelet count / cmm	261.70	55.337	158.00	356.00	-0.123
10	CD 34^+ %	0.651	0.247	0.39	1.14	1.18
11	$CD34^+$ count	20.20	7.269	10.00	35.00	0.800

Skew factor of all participant were between (+,- 3)

Table (2) Mean + SD, Range and Skew for Fitness and Skill Variables (n=10)

S	variable	Mean	SD	Range		SK
				small	big	
1	Fitness	64.6000	9.27601	53.00	77.00	.072
2	Back scissor	.4000	.39441	.00	1.00	0.407
3	Star Jump	1.7500	.85797	1.00	3.50	.990
4	Bike Jump	1.0500	.72457	.0	2.50	.608
5	Tone	.3500	.52967	.00	1.50	1.444
6	Grand Jete	.8500	.52967	.00	1.50	0.42
7	Leap	.6000	.39441	.00	1.00	-0.407
8	Tuck Jump	1.8500	.74722	1.00	3.00	0.140
9	Attitude	.0000	.00000	.00	.00	0.0
10	Age	19.6000	.84327	19.00	21.00	1.001

Skew Factor of all parameters were between (+,- 3)

Tools and devices used:

1. Stopwatch
2. Different weights
3. Step box
4. Cassette and music discs
5. Syringes, cotton, spirit
6. Test tubes
7. Balance weight, Restameter
8. Freezer to store the samples
9. EDTA anticoagulant
10. Coulter counter for blood components estimation
11. Centrifuge for separation of plasma and blood content
12. Flow cytometer to determine $CD34^+$
13. Monoclonal antibodies against human $CD34^+$
14. Fluorochrome – conjugated antibodies
15. Vortex for shaking test tubes
16. Cell pلاllet
17. Pulsemeter
18. Ice box

Blood components $CD34^+$, Rbcs, Wbcs, platelets, hemoglobin, MCH, Hematocrit, MCV, MCHC, RDW. Fitness and skill variables (Fitness, Back Scissor, Star jump, Bike jump, Tonent, Grand jete, Leap, Tuck jump, Attitude).

Statistical Analysis:

Students "t" test were used to test the differences before and after exercise program. The non-parametric Man Whitney (Wilcoxon rank sum test) was used to compare between pre and post exercise program variables together with change %. For descriptive data the median (lowest value –

highest value) was displayed. For parameters with non-normal distribution, non- parametric spearman correlation coefficients were used. An α level of 0.05 was used to indicate statistical significance.

Table (3) Time distribution of exercise program

No	Phase	Time
1	Warm up	5-7 min
2	Main Phase General exercise steps Positive rest Bar exercise Positive rest Skill training	50-78 min 20-30 min 1-2 min 10-20 min 1 min 18-25 min
3	Cool down	5 min

Before the main experiment the researchers have done the following:
The graduation of the time of bout of exercise from 60-90 min.
Precision of intensity of exercise by using the following equation
Max heart rate = 220 –age.

- Precision the median intensity for each participant 50 % of Max heart rate.
- Precision of sub maximum intensity 75 % of Maximal heart rate.

3- Follow the positive rest by deep respiration during the main phase of the exercise bout.

Table (4) Mean+ SD of $CD34^{+}$and blood components in pre and post exercise program of group sample (n=10)

S	Variables	measure	Rank	different		Z value	P value
				direct	Number		
1	Hemoglobin gm/dl	Pre Post	2.00 7.00	- + =	3 7 0	-2.194	0.028
2	Red cell count mil/cmm	Pre Post	7.25 4.33	- + =	4 6 0	-0.153	0.878
3	MCH pg	Pre Post	0.00 5.50	- + =	0 10 0	-2.814	0.005
4	RDW	Pre Post	1.00 5.50	- + =	1 8 1	-2.552	0.011
5	Hematocrit %	Per Post	4.33 6.00	- + =	3 7 0	-1.479	0.139
6	MCV fL	Pre Post	0.00 6.00	- + =	0 10 0	-2.807	0.005
7	MCHC gm/dl	Pre Post	3.50 6.00	- + =	2 8 0	-2.094	0.036
8	Total leucocytic count/ cmm	Pre Post	5.00 5.63	- + =	2 8 0	-1.785	0.074
9	Platelet count / cmm	Pre Post	6.17 4.50	- + =	6 4 0	-0.968	0.333
10	CD 34+ %	Pre Post	0.00 5.50	- + =	0 10 0	-2.803	0.005
11	Count CD34+	Pre Post	0.00 5.50	- + =	0 10 0	-2.803	0.005

Table (4) indicated significant change between before and after the program in the variables (1,3,4,6,7,8,10,11), where number (2,5,9) were non significant.

Table (5) Change rate % of pre and post exercise program of participants in $CD34^+$ and blood components.(n=10)

S	variables	Mean pre	Mean post	Change %
1	Hemoglobin gm/dl	12.37	12.72	2.83
2	Red cell count mil/cmm	4.43	4.418	-0.34
3	MCH pg	28.07	28.94	3.10
4	RDW	12.89	13.40	3.96
5	Hematocrit %	36.87	37.51	1.74
6	MCV fL	83.66	85.30	1.96
7	MCHC gm/dl	33.52	33.91	1.16
8	Total leucocytic count/ cmm	7.57	8.66	14.40
9	Platelet count / cmm	261.7	247.00	-5.62
10	CD 34+ %	0.651	2.29	251.77
11	CD34+ count	20.20	62.20	207.92

Table (5) Showed that change % of pre and post program between (-5.62, 251.77).

Table (6) Statistical difference between pre and post exercise program of participants in physical, skill variables. (n=10)

S	Variables	Measure	Rank	different		Z value	P value
				direct	Number		
1	Fitness	Pre Post	55 0	- + =	0 10 0	-2.805	0.005
2	Back scissor	Pre Post	36 0	- + =	0 8 2	-2.539	0.011
3	Star Jump	Pre Post	36 0	- + =	0 8 2	-2.636	0.008
4	Bike Jump	Pre Post	28 0	- + =	0 7 3	-2.414	0.16
5	Tonent	Per Post	55 0	- + =	0 10 0	-2.842	0.004
6	Grand Jete	Pre Post	55 0	- + =	0 10 0	-2.831	0.005
7	Leap	Pre Post	55 0	- + =	0 10 0	-2.848	0.004
8	Tuck Jump	Pre Post	21 0	- + =	0 6 4	-2.214	0.027
9	Attitude	Pre Post	55 0	- + =	0 10 0	-2.814	0.005

Table (6) indicated significant change between before and after the program in the variables (1,2,3,5,6,7,8,9), where number (4) was non significant.

Table (7) Change rate % of pre and post exercise program of participants physical, skill variables. (n=10)

S	variables	Mean pre	Mean post	Change %
1	fitness	64.6000	95.400	47.68
2	Back scissor	.4000	1.500	275.00
3	Star Jump	1.7500	2.550	45.70
4	Bike Jump	1.0500	1.950	85.70
5	Tonent	.3500	1.950	457.14
6	Grand Jete	.8500	2.000	135.29
7	Leap	.6000	2.050	241.67
8	Tuck Jump	1.8500	2.550	37.84
9	Attitude	.0000	1.450	145.00

Table (7) Showed that change % of pre and post program between (37.84, 457.14).

Results and Discussion:

There have been a few studies published regarding the effect of exercise program on $CD34^+$. Our data (Table 4,5) revealed a significant increased $CD34^+$ after an 8 weeks exercise program. These result were in accordance with that of Zaldivar et al, (2007) and Jung et al,(2008), while Laufs et al,(2005) indicated a decreased $CD34^+$ after one day of wheel running exercise and significantly increased after 7 days of exercise. Sarah Witkouski (2008) indicated that chronic exercise training may improve antioxidant capacity and may be one mechanism by which exercise improves stem cell function.

Asaf et al, (2008) described how stress- induced stem cell recruitment in sport activities, which induce the egress of immature progenitors and arise in progenitors in the blood. They added that once stress conditions arise, the process of stem cell recruitment is dramatically amplified, by increasing osteoclast activity in the bone marrow endosteal region, and participation of chemokines, cytokines and proteolytic enzymes, with increased SDF_1 expression and its receptor $CXCR4^+$, together with catecholamine and its catecholamine receptors, they enhanced together the stimulation and proliferation and mobilization of stem cell to circulation.

In recent years, human stem cells mobilization has become the preferred source of repopulating stem cells for clinical transplantation protocols, due to the higher yield of immature cells, shorter time frame to reach successful repopulation, and reduced technical intervention and pain advantages compared to harvesting cells from bone marrow or cord blood (Lapidot and Petit, 2002). This was in accordance with the proposed of the researchers who aimed to a natural increased in stem cells through a proposed exercise program.

The data presented in table (4, 5) indicated an increased Hemoglobin concentration (change % + 2.83), MCH (3.10 %), RDW (3.96 %), Hematocrit (1.74 %), MCV (1.96 %), MCHC (1.16 %) together with an increased Leucocytic count (Wbcs)(14.40 %) and an enormous increased in $CD34^+$ count (207.92%). There was a negative change % in case of Rbcs count (-0.34 %) and plateltes counts (-5.62 %).

This was in agreement with Hoellriegel et al,(2008), Bonsignore et al, (2002). They reported that physical exercise training for 2-3 months result in: 1) increase in number and functional capacity of hematopoetic stem and progenitor cells. 2) angmentation of capillary density in the skeletal muscle. 3) improvement of hemodynamtic parameters and exercise capacity.

Guyton and Hall (2006) added that a key requirement of cardiovascular function in exercise is to deliver the required oxygen and other nutrients to the exercising muscles, for this purpose, the muscle blood flow increases drastically during exercise also due to capillary density in the skeletal muscle as a result of adaptation to long term exercise program.

Robergs and Roberts (2000) also added that blood consists of several types of cells, which all emanate from stem cells located in bone marrow, the original stem cell can differentiate into precursors of White blood cells (leucocytes), Red blood cells or Platelets, the stimulation of erythropoesis (blood cells) is under control of the hormone erythropoietin.

Shaimaa El Said (2012) reported that the increase of leucocytes due to chronic exercise might be due to secretion of epinephrine and cortisol hormones which in turn increase Wbcs and immunity

of sports women. As for the small decrease in Rbcs count after exercise program (-0.34%), it might be caused due to destruction of some Rbcs due to exercise on a hard ground, leading to the lower count of Rbcs in 4 participants in contrast to the increased Rbcs in 6 girls. The researchers also noted a decrease but within the normal values (150.000-300.000) in the number of Platelets after exercise program (-5.62), it might be caused due to several mechanism, but the bone marrow may be able to compensate as stated by Waterbury (2007), that thrombocytopenia (a mild decrease in platelets) is present in cases of disseminated intravascular coagulation, and platelets return to normal slowly, or due to immune thrombocytopenia caused by infections or due to drug administration of participants.

The increased hemoglobin, hematocrit, MCV, MCH, MCHC, in this investigation after exercise program denotes a positive result as the parameter(Hb) carry four molecules of oxygen, Hb combine with oxygen to form oxyhemoglobin, each gram of Hb combine with 1.33 ml of oxygen. The increased parameters might also increase performance. As for the decreased platelets, which are minute oval non nucleated granules. They are formed in the bone marrow from megakariocytes.

Barret et al, (2010) reported that normal count of platelets is between 150.000 – 500.000 /cmm and increased during digestion, muscular exercise, high altitude and pregnancy and decreased during menstruation (period).

Data presented in table (6) revealed a significant increase of physical and skill variables and the change % of different parameters were between (37.84% and 457.14%) after exercise program. The researchers indicated that the significant increase of physical and skill variables might be due to the positive effect of exercise program, and the different components of the specificity principle applied. Robergs and Roberts (2000) reported that the process of training is performed to improve fitness. Training involves the organized sequence of exercise that stimulates improvements, or adaptations, in anatomy and physiology. Depending on the quality of training and the duration of intervals between exercise sessions, these training – induced improvements are developed and retained. In most circumstances, improved tolerance results in improved exercise performance. Therefore knowledge of the types, intensities, durations and frequencies of exercise required to optimize training adaptations is essential. This knowledge is acquired from the study of skeletal muscle energy metabolism and from understanding the principles and terminology of training. (Dimeo, 1997, Mock et al, 1997, Nieman 1995). Gillen et al, (1991) stated that athletes can increase cardiorespiratory and muscular endurance, or muscle power, by performing a combination of training protocols. Strength gains can be increased when dynamic rather than isometric contractions are used and when eccentric contractions are incorporated into the training program. Similar increases in strength occur from variable resistance and constant resistance exercise.

From the above,it is clear that the three hypothesis have been realized

Conclusion:

It may be concluded that:

- $CD34^{+}$ counts increased after exercise program, it might be the preferred source of repopulating stem cells.
- The increased blood cell counts except platelets count indicated an improvement of hemodynamic parameters.
- The increase of physical and skill variables indicated a positive effect of exercise program, and the different components of the specificity principle applied, taking account the individual variations.

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