The Effect of Aerobic and Anaerobic Exercise Bouts on CD^{34+} Stem Cells and Some Physiological Parameters

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Abstract: Aerobic exercise draws energy mainly from biochemical processes requiring oxygen, whereas anaerobic exercise draws energy from processes not requiring oxygen.20 healthy male athletes aged (18-24 yrs.) were recuited for this study. Healthy low active males and BMI matched participants (n=10) aged (20-22 yrs.) wererecuited as controls. Aerobic and anaerobic testing was performed on a cycle ergometer. The testing wasa modification of AstrandRhyming protocol forVo2max. Pulserate estimation,Rbcs,Wbcs, HB and hematocrit were estimated using coulter counter. Lactateby accusport, CD34+ stem cells were determined by flow cytometry. Results indicated:VO2 max was increased in case of aerobic exercise bout compared to anaerobic one.Lactate concentration was decreased in case of aerobic exercise bout compared to anaerobicone.Hb, Rbcs,Wbcs and hematocrit were increased after both exercise bouts.CD34+ stem cells were increased in case of anaerobic exercise bout than aerobic one. It is concluded that Vo2 max increased in case of aerobic exercise bout compared to anaerobic one due to the longer period of cycling. Lactate concentration was decreased in case of aerobic exercise bout compared to anaerobic one due to the higher intensity expressed in anaerobic bout leading to decrease oxygen .CD^{34+} HPC counts were increased in peripheral blood of anaerobic exercise bout than aerobic one due to stress induced by anaerobic exercise bout .
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Key words: Aerobic and anaerobic exercise bouts, CD34+stem cells, physiological parameters.

1. Introduction

Jogging is an endurance exercise; in contrast, resistance exercise involves short periods of contractile activity against high resistance. Weightlifting is a resistance exercise. Sprint exercise consists of short periods of maximal contractile activity against low resistance. A competitive 50m swim is a sprint exercise (Mougios, 2006). He also added that an alternative way of describing exercise type is by the terms aerobic and anaerobic. Aerobic exercise draws energy mainly from biochemical processes requiring oxygen, whereas anaerobic exercise draws energy from processes not requiring oxygen.

Although exercise is considered a physiological stimulus for cell release by the bone marrow (Brenner et al., 1998), surprisingly few data are available on circulating hematopoietic precursors in athletes. Erythrocyte production was studied relative to athletes anemia (Szygula, 1990) and to assess the effects of intermittent hypoxic exposure on exercise performance (Baily and Davies, 1997). Conversely, little is known of the effects of exercise on myeloid precursors. For many years, it was reported that colony forming cells in peripheral blood increased after a short and intense exercise bout in normal subjects (Harrett et al., 1978), but a detailed characterization of hematopoietic precursors in well trained subjects was never obtained. (Bonsignore et al., 2002).

The rationale to study myeloid precursors in athletes is that intense and prolonged exercise increases white blood cell (WBC) and neutrophil count (Brenner et al., 1998 and Nieman, 1997).

Stem cells are not specialized and incomplete division was no similarity of any specialized cell. But are able to form an adult cell is divided after several divisions in appropriate circumstances, and the importance of these cells comes from being unable to form any kind of specialized cells after grow and develop into cells is required.(Laufs et al,2004).

Thus, the stem cells in turn depends on the so-called «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 types of tissue, 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:Embrionicstem cells, and adult stem cells. (Rehman etal, 2004)Barrett et al, (2010).

In healthy moderately trained subjects an acute bout of moderate to hard intensity endurance exercise has been shown to increase EPC number, EPC migration and colony forming units. (Laufs et al., 2005)

The Aim of this study Is to reveal:

1- The role played by aerobic and anaerobic exercise bouts on CD^{34+}stem cellsdetermination.
2- The role of aerobic and anaerobic exercise bouts on some physiological parameters.

2. Material and Methods

Participants:

20 healthy male athletes aged (18-24 yrs.) with a training history of (4-9yrs) were recuited 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 nonsmokers, 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 11 receptor antagonists, ACE inhibitors; peroxisome proliferators activated receptor (PPARα) 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 weight 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.

Aerobic and anaerobic testing was performed on a cycle ergometer with physician monitoring. Heart rate and blood pressure were monitored continuously throughout the test. The testing was a modification of Astrand Rhyming protocol, until the subject exhaustion.

Maximal oxygen consumption (VO_{2max}) is the maximal rate at which the body can consume oxygen during exercise (Davis et al., 1976). The test of maximal oxygen consumption is an example of both low and high intensity exercise (50 watt increment, 3 min stage protocol in aerobic exercise 25 watt each as for anaerobic exercise 100 watt increment, 30 second stage protocol by adding 50 watt each). The incremental exercise is used by bicycle ergometer against increasing intensities until volitional fatigue. The Astrand Rhyming nomogram for estimating Vo_{2max} to use the nomogram for cycle ergometry exercise, a line is drawn connecting the gender specific heart rate to the specific workload (kg/min). When this straight line intersects the diagonal Vo_{2max} line represents the Vo_{2max} value.

The predicted VO_{2max} value is obtained by connecting the point on the VO_{2max} scale with the corresponding point, on the pulse rate scale.

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 non-nucleated, 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 esonophil.(Guyton and Hall20006).

All blood cells were counted using coulter counter which is easy to read numerical presentation.

Lactate analysis was performed by using accusport before and after the test by venipuncture:

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 EDTA-coated 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 CD^{34+} 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:

IOTest CD34-PE:

Use this fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the CD^{34+} antigen present in human biological samples using flow cytometry.

Principle

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes.

Specific staining of the leucocytes is performed by incubating the sample with the IOTest reagent. The red cells are then removed by lysis and the leucocytes, which are unaffected by this process, are analyzed by flow cytometry.

The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage depending on the application chosen by the user.

The fluorescence of the delimited cells is analyzed in order to distinguish the positively stained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

Procedure:

Note: The procedure below is valid for standard applications. Sample and/or VersaLyse volumes for certain Beckman Coulter applications may be different. If such is the case, follow the instructions on the application's technical leaflet.

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed in the presence of the isotopic control (Ref. A07796).

1- Add 20 µL of specific IOTest conjugated antibody to each test tube, and 20 µL of the isotypic control to each control tube.
2- Add 100 µL of the test sample to both tubes. Vortex the tubes gently.
3- Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
4- Then perform lysis of the red cells, if necessary, by following the recommendations of the lysis reagent used. As an example, if you wish to use VersaLyse (Ref. A09777), refer to the leaflet and follow preferably the procedure called "with concomitant fixation", which consists of adding 1 Ml of the "Fix-and-Lyse" mixture prepared extemporaneously. Vortex immediately for one second and incubate for 10 minutes at room temperature, protected from light. If the sample does not contain red cells, add 2 mL of PBS.
5- Centrifuge for 5 minutes at 150 x g at room temperature.
6- Remove the supernatant by aspiration.
7- Resuspend the cell pellet using 3 mL of PBS.
8- Repeat step 5.
9- Remove the supernatant by aspiration and resuspend the cell pellet using:
   - 0.5 Ml or 1 Ml of PBS plus 0.1% of formaldehyde if the preparations are to be kept for more than 2 hours and less than 24 hours. (A 0.1% formaldehyde PBS can be obtained by diluting 12.5 µL of the IOTest 3 Fixative Solution (Ref. A07800) at its 10X concentration in 1 Ml of PBS).
   - 0.5 Ml or 1 Ml of PBS without formaldehyde, if the preparations are tobe analyzed within 2 hours.

Note: In all cases, keep the preparations between 2 and 8°C and protected from light.

Height and weight were recorded and body mass index, BMI (kg/m2) was calculated for all subjects. A BMI score less than 20 is considered underweight, 20 to 24,9, is considered desirable, 25 is considered overweight, and greater than 30 is considered obese.

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 non-parametric Mann Whitney utest (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 were 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.

3. Results

Subjects characteristics:

20athletes and 10 low active control males participated in the study. Groups were matched for age,

weight and height (table 1). Also for BMI non-significant changes in basic characteristics, to compare athletes and control males. Pulse rate and VO_{2max} 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.Data for CD^{34+} number. There were significant difference between athletes after anaerobic exercise bout compared to aerobic and control indicated in Table (2).Data for CD^{34+} number. There were significant difference between athletes after aerobic and anaerobic exercise bout as indicated in Table (3).Lactate Revealed a significant increase after anaerobic bout values are means +SE $P<0.05$.) Revealed NS change in case of participants of control and athletes groups at rest in hematological values in Table (4). Revealed a significant change in participants after aerobic and anaerobic bout of exercise in hematological values $P< 0.05$ in Table (5).VO_{2max} (mL/kg/min) results indicated an increased value between the healthy sedemtary participants and after aerobic exercise bout and anaerobic one in Table (6).

4. Discussion:

The data presented indicated that lactate concentration in Table (1) was in the normal range with non-significant changes in both groups (control and athletes).

After aerobic exercise bout and anaerobic one, the concentration of lactate showed a higher value in case of anaerobic exercise compared to aerobic bout Table (3).The increased lactate may be due to higher intensity expressed in anaerobic bout leading to decrease oxygen.

In case of intense exercise, which can be defined as any intensity that exceeds an individual's capacity to maintain a steady state condition, ATP regeneration must be met by creatine phosphate hydrolysis and by glycolysis terminating in the production of lactate and the eventual development of acidosis. Intense exercise can be performed in many ways, such as the intense exercise of sprint, swimming, cycling, or in incremental exercise (Robergs and Roberts, 1997), lactate and protons leave the muscle fiber by a similar mechanism of incremental exercise. Roth and Brooks (1989) have presented the kinetics of a lactate transporter and have shown that it is a saturated transport process. It is believed that protons leave the muscle in combination with the lactate transporter (MCTs) via facilitated transport (Stanley et al., 1985) which accounts for similar changes in blood lactate and acidosis during intense exercise.

During prolonged exercise, muscles and blood lactate concentration peak a few minutes after the start of exercise of moderate to low intensity and drop slightly as exercise continues. After the end of exercise both return to baseline gradually (Fitts, 2004).

Vo_{2max}. values range from those of persons extremely low capacities, such as chronically ill individuals (< 20 ml/ kg/min) to those of well-trained and elite endurance athletes (> 80ml/ Kg/ min) (Robergs and Roberts, 1997). They also added that the factors that combine to influence Vo_{2max} are a high proportion of slow twitch motor units, high central and peripheral cardiovascular capacities, and the quality and duration of training. Having more slow twitch muscle fibers increases the oxidative capacity of the muscle (Jacobs, 1983*)*.He stated that muscle motor unit proportions are genetically determined and therefore a person's abilities to respond to endurance training and increase to Vo_{2max} have important genetic constraints. This opinion is in accordance with the results in table (6), as aerobic exercise bout of participants a higher Vo_{2max} than control and anaerobic participants. As an increased mitochondrial volume would also provide skeletal muscle with the ability to increase maximal oxygen consumption. However, cardiovascular adaptation are also involved in increasing Vo_{2max} after training, and muscle adaptations should not be viewed as the role determinant of Vo_{2max}. As different training strategies influence the values of Vo_{2max}., and it appears that the type and quality of training are also important. The extent of improvement in Vo_{2max} depends on the value of Vo_{2max} before training. (Robergs and Roberts 1997).

The hemoconcentration may be the main cause of the increase blood parameters of Rbcs, Wbcs, Hb and Hematocrit (Table 4,5) after the aerobic and anaerobic exercise bout, and the increased blood parameters could be caused by the stress induced by physical activities (Montain and Coyle 1992).

The results in Table (2,3) indicated that CD^{34+} increased after exercise bouts. The increased haematopoietic stem cells CD^{34+} revealed a positive results specially the anaerobic bout who were subjected to stress more than the athletes subjected to aerobic bout.

Previous studies have shown that an acute bout of exercise increases the number of bone marrow derived endothelial cells in the blood (Shaffer et al., 2006 and Vancraenenbroeck et al., 2008 and Amany and Mohamed, 2011).

This is consistent with our data, as aerobic and anaerobic bout of exercise revealed an increase in CD^{34+}(SC) as shown in table (3), anaerobic bout of exercise was more prominent in increasing CD^{34+} (SC).

Table (1): Basic characteristics

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 ±SE P<0.05
BMI = body mass index

Table (2): CD^{34+} in case of aerobic and anaerobic exercise bout and control

	CD^{34+}						ANOVA	
	Range			Mean	±	SD	F	P-value
GI (anaerobicexercice bout) n=10	227	-	366	284.5	±	51.5		
GII (aerobicexercice bout) n=10	144	-	216	173	±	22.7	26.85	0.001
Control n=10	140	-	210	172	±	24.1		

Tukey's test

GI (anaerobic) VS GII (aerobic)	GI (anaerobic) VS control	GII (aerobic) VS control
0.001	0.001	0.999

Table (2) There were significant change in CD^{34+} for the favor of anaerobic exercise bout.

Hematopoietic stem cells:

Data for CD^{34+} number. There were significant difference between athletes after anaerobic exercise bout compared to aerobic and control indicated in table (2).

Table (3): Revealed data of CD^{34+} (SC) and lactate after exercise bout aerobic and anaerobic

Variable	Aerobic exercise bout			Anaerobic exercise bout		
CD^{34+} (HPc) cells	173	±	22.7	284.5	±	51.5
Lactate (mmol/L)	3.2	±	0.4	5.6	±	0.8

Table (3) Data for CD^{34+} number. There were significant difference between athletes after aerobic and anaerobic exercise bout as indicated in CD^{34+} for the favor of anaerobic exercise bout also Lactate revealed a significant increase after anaerobic bout values are means +SE P<0.05.

Table (4):Haematological values of RBcs, Wbcs, hematorit (PCV) and hemoglobin in control and athletes at rest.

Variable	Control			Athletes			Sig
Rbcs (million/mm3)	4.9	±	0.9	4.1	±	0.6	NS
Wbcs (thousands/ mm3)	4.8	±	0.7	5.9	±	0.8	NS
Hb (g/dL)	12.8	±	0.8	14.2	±	0.9	NS
Hematocrit (%)	42	±	3.2	42.1	±	2.7	NS

Table (4) Revealed NS change in case of participants of control and athletes groups at rest in hematological values. P< 0.05 mean ± SE.

Table (5):Hematological values of Rbcs, Wbcs, Hb, and Hematocrit (PCV) in aerobic and anaerobic exercise bout.

Variable	Aerobic			Anaerobic			Sig
Rbcs (million/mm3)	4.8	±	0.3	5.1	±	0.2	S
Wbcs (thousands/ mm3)	5.9	±	0.5	6.5	±	0.4	S
Hb (g/dL)	13.8	±	0.9	15..2	±	0.8	S
Hematocrit (%)	43	±	1.2	45	±	1.1	S

Table (5): Revealed a significant change in participants after aerobic and anaerobic bout of exercise in hematological values P< 0.05

Table (6): The variation in VO_{2max} for participants healthy sedentary, aerobic and anaerobic exercise bout.

Participants	VO_{2max} (mL/kg/min)		
Healthy sedentary (mL/kg/m)	36	±	1.7
Aerobic exercice (mL/kg/m)	57	±	2.4
Anaerobic exercice (mL/kg/m)	54	±	2.5

The results are expressed as mean ± SE ($P<0.05$).

Table (6) VO_{2max} (mL/kg/min) results indicated an increased value between the healthy sedentary participants and after aerobic exercise bout and anaerobic one.

The number of circulating EPSs likely represents the balance between liberation of EPCs from the bone marrow and incorporation at the level of the vessel or differentiation. Laufs et al.,(2005) demonstrated that CD^{34+}/KDr^{+} increase after 30 minutes of high intensity running in healthy participants, but returned to resting levels by 24 hours following exercise. It can be speculated that in healthy regularly exercising individuals, by 24 hours following exercise.

Also, it was reported that human subjects undergoing exhaustive dynamic exercise revealed an increased EPC counts in the peripheral blood (Laufs et al., 2005 and Rehman et al., 2004).

Giuseffe et al., (2005) reported an increased CD^{34+}stem cells and reticulocytes after supramaximal exercise, they added that this increase was unlikely to depend on changes in blood or plasma volume, since these were much smaller than changes in cell counts. either been incorporated for endothelial repair, neovascularization or have undergone differentiation.

Ewa and Pawet, (2007) reported that a decrease in the blood supply to a bodily organ or tissue, caused by constrictor or obstruction of the blood vessels, is a common cause of ischemia. This process is probably responsible for the use of EPCs in postnatal vascular growth and remodeling. In the study performed by Adams et al., (2004), patients with stable CAD were subjected to the single-exercise stress test to compare peripheral blood EPC counts before and after the experiment. It was found that the peripheral blood EPC count was increased significantly in ischemic patients within 24-48 hours after exercise. They observed that an increase in EPC levels was accompanied by an elevation of VEGF concentration in plasma in these patients. These results confirmed that VEGF is a significant factor responsible for EPC mobilization from bone marrow to peripheral blood (Adams et al., 2004).

Conclusion

It may be concluded that:

- Vo2 max increased in case of aerobic exercise bout compared to anaerobic one due to the longer period of cycling.
- Lactate concentration was decreased in case of aerobic exercise bout compared to anaerobic one due to the higher intensity expressed in anaerobic bout leading to decrease oxygen .
- Hb, Rbcs, Wbcs and hematocrit value were increased after aerobic and anaerobic exercise bout.
- CD^{34+} HPC counts were increased in peripheral blood of anaerobic exercise bout than aerobic one due to stress induced by anaerobic exercise bout.

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