Effect of different exercise intensities on CD^{34+} stem cells and physiological variables parameters

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Abstract: Aerobic exercise draws energy mainly from biochemical processes requiring oxygen, whereas anaerobic exercise draws energy from processes that do not require oxygen. Twenty 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 exercises were performed on a cycle ergometer. The testing was a modification of the A Strand Rhyming protocol for Vo2max. Pulse rate estimation, RBCs, WBCs HB and hematocrit were estimated using a coulter counter. Lactate by accusport, CD34+ stem cells were determined by flow cytometry. **Results indicated:** Vo2 max was higher during aerobic compared to anaerobic exercise. Lactate concentration decreased in aerobic compared to anaerobic exercise bouts. H, RBCs, WBCs, and hematocrit increased after both types of exercise bouts. The increase in CD34+ stem cells during anaerobic exercise bouts was greater than it was during aerobic exercise bouts. It is concluded that these findings deserve further study.
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1. Introduction:

Jogging is an endurance exercise. In contrast, resistance exercise, such as weight lifting, involves short periods of contractile activity against high resistance. On the other hand, sprint exercise consists of short periods of maximal contractile activity against low resistance. For example, a competitive 50 m swim is a sprint exercise (Mougios, 2006).

He also suggested an alternative way to describe exercise type using the terms aerobic and anaerobic. Aerobic exercise draws energy mainly from biochemical processes requiring oxygen, whereas anaerobic exercise draws energy from processes that do not require 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 athlete's anemia (Szygula, 1990) and to assess the effect of intermittent hypoxic exposure on exercise performance (Baily and Davies, 1997). Conversely, little is known about the effect 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 counts (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, stem cells, in turn, depend on the so-called "old fetal" of the body. These are stem cells that have the ability to become anything. Then, there are the stem cells "college ability" that can become one of several types of tissue. There are also adult stem cells that can proliferate to create a special texture to the body such as the liver, bone marrow, or skin, etc. Thus, with each step toward adulthood, the successes achieved by the stem cells are narrower, leading to specialization. During adulthood, does not generate liver cells, but other liver cells, skin cells, generate another. However, recent research indicates that the number 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 two forms of stem cells: embryonic and adult stem cells. (Rehman *et al.,* 2004; Barrett *et al.,* 2010).

In healthy, moderately trained subjects, an acute bout of moderate to high 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 the effect of physical activity on the support and enhance the natural behavior of stem cells in the body:

1-The role played by aerobic and anaerobic exercise bouts on CD34+ stem cells determination.

2-The role of aerobic and anaerobic exercise bouts on some physiological parameters.

2. Material and Methods

Participants:

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

All participants were nonsmokers, non-diabetic and free of cardiovascular, lung, and liver disease. Participants did not take any medications that could affect EPC number or function. These included 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 Arish institutional review 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 assess and obtain samples of blood for analyses and BMI testing.

They were given a weight data log and instructed to weigh themselves in the morning and evening and record their weight in the log. All participants refrained from caffeine, vitamins, and any medications 48 hours prior to testing. Participants were provided with a log book to record their food intake for the three days prior to testing.

Aerobic and anaerobic tests were performed on a cycle ergometer under the supervision of a physician. Heart rate and blood pressure were monitored continuously throughout the tests. The testing was a modification of A Strand Rhyming protocol, until the subject exhaustion.

Maximal oxygen consumption (VO2max) 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 watts each as for anaerobic exercise 100-watt increment, 30 second stage protocol by adding 50 watts each). The incremental exercise is used by bicycle ergometer against increasing intensities until volitional fatigue. The A Strand Rhyming nomogram for estimating Vo2max to use the nomogram for cycle aerometry exercise, a line is drawn connecting the gender specific heart rate to the specific workload (kg/min). When this straight line intersects the diagonal Vo2max, the line represents the Vo2max value.

The predicted VO2max value is obtained by connecting the point on the VO2max scale with the corresponding point on the pulse rate scale.

RBCs, WBCs Hb and hematocrit values were estimated using a coulter counter.

The human erythrocyte, which is the mature unit of the red blood corpuscle, is a circular, elastic non-nucleated, biconcave disc, whose primary function is to transport hemoglobin.

Hemoglobin is a protein made up 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 cells when a known volume of whole blood is centrifuged at a constant speed for a constant period.

White blood corpuscles (leukocytes) include all white cells of the blood: lymphocyte, monocyte neutrophil and basophil and eosinophil. (Guyton and Hakk, 2006).

All blood cells were counted using a coulter counter, with which numerical values may be easily read.

Lactate analyses were performed using accusport before and after the tests by venipuncture.

Circulating progenitor cell number:

CD34+ (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 cells were immunoassayed with monoclonal antibodies against human CD34+ for each group of analyses, and one set of control tubes for machine calibration was generated. Flow cytometry

was performed in the specialized laboratory Of (NSA lab).

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 were as follows:

IOTest CD34-PE:

The use of the Fluor chrome-conjugated antibody permits the identification and numeration of cell populations expressing the CD34+ 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 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 Versa Lyse volumes for certain Beckman Coulter applications may be different. If such is the case, instructions on the application's technical leaflet are followed.

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 isotypes 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, following the recommendations of the lysis reagent used. As an example, if you wish to use Versa Lyse (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 Phosphate buffer saline.

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 to be 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 Analyses

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

3. Results

Subjects characteristics:

Twenty players 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 were examined to compare Handball player and control males. Pulse rate and VO_{2max} showed significant changes (Table 1), i.e., expected Handball players had lower pulse rates compared to controls. Physical

activity questionnaire data revealed that Handball player exercised an average of 5±0.5 days a week for 5±0.2 years.

Control group participants did not exercise regularly, nor did they have a recent history of physical activity.

Table (1): Basic characteristics

Variable	Handball players n=20	Control n=10			Sig.
Age (yr.)	21.6±1.83	20.6	±	0.89	NS
Height (cm)	163±2.18	168.1	±	1.12	NS
Weight (kg)	73±2.16	69	±	1.5	NS
BMI	21±1.3	25	±	2.5	NS
Pulse rate (count/m)	68±2.3	74	±	2.1	S
VO_{2max} (ml/kg)	52±1.8	40	±	1.9	S
Lactate (mmol/L)	1.1±0,.02	1.5	±	0.05	NS

Values are means ±SE P<0.05 BMI = body mass index

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

	CD^{34+}					ANOVA
	Range			Mean	F	*P*-value
GI (anaerobic exercise bout) n=10	230	-	379	290.5±54.8	29.35	0.001
GII (aerobic exercise bout), n=10	138	-	213	192±32.9		
Control, n=10	120	-	190	159±22.4		
Tukey's test						
GI (anaerobic) VS GII (aerobic)	GI (anaerobic) VS control			GII (aerobic) VS control		
0.001	0.001			0.999		

Hematopoietic stem cells:

Data for CD^{34+} number. There were significant differences between athletes after anaerobic exercise bout compared to aerobic and the control (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 differences between athletes after aerobic and anaerobic exercise bouts (Table 3).

Lactate increase significantly after anaerobic bouts; values are means +SE *P*<0.05.

Table (4): Hematological values of RBCs, WBCs, Hematocrit (PCV) and hemoglobin in control and athletes at rest.

Variable	Control			Athletes			Sig
RBCs (million/mm3)	5.9	±	0.9	4.1	±	0.6	NS
WBCs (thousands/ mm3)	4.8	±	0.6	6.3	±	0.9	NS
HB (g/dL)	12.8	±	0.8	15.2	±	0.9	NS
Hematocrit (%)	41	±	3.1	44.2	±	3.1	NS

P< 0.05 mean ± SE.

Table (4) Revealed NS change in case of participants of control and athlete's groups at rest in hematological values.

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	±	0.5	6.1	±	0.2	S
WBCs (thousands/ mm3)	5.3	±	0.7	7.2	±	0.6	S
HB (g/dL)	14.2	±	0.9	16.1	±	0.9	S
Hematocrit (%)	43	±	1.4	46	±	1.6	S

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

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

Participants	VO_{2max} (ml/kg/min)		
Healthy sedentary (ml/kg/min)	36	±	2.3
Aerobic exercise (ml/kg/min)	58	±	2.6
Anaerobic exercise (ml/kg/min)	53	±	2.3

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 both aerobic and anaerobic exercise bouts.

4. Discussion:

The data presented indicates that lactate concentrations (Table 1) were in the normal range with nonsignificant changes in both groups (control and athletes).

The concentration of lactate was higher following anaerobic exercise than it was following aerobic exercise (Table 3). Such an increase in lactate may be a result of a greater decrease in oxygen during intense anaerobic exercise.

In the 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) 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 gradually return to baseline values (Fitts, 2004).

Vo 2max. values range from those of persons with 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 Vo2max are a high proportion of slow twitch motor units, high central and peripheral cardiovascular capacities, and the quality and duration of training. Having slower 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 ability to respond to endurance training and increase to Vo2max has important genetic constraints. This opinion is in accordance with the results in Table 6, i.e., Vo2max was higher in those who participated in aerobic exercise bouts compared to the controls and those who participated in anaerobic exercise bouts. An increase in mitochondrial volume would also provide skeletal muscle with the ability to increase maximal oxygen consumption. However, cardiovascular adaptation is also involved in increasing Vo2max after training, and muscle adaptations should not be viewed as the sole determinant of Vo2max. Different training strategies can influence the values of Vo2max, and it appears that the type and quality of training are also important. The extent of improvement in Vo2max depends on the value of Vo2max before training. (Robergs and Roberts, 1997).

The hemoconcentration may be the main cause of the increase blood parameters of RBCs, WBCs, He, and Hematocrit (Tables 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) indicate that CD34+ increased after exercise bouts. The increased haematopoietic stem cells CD34+ revealed positive results, especially the anaerobic bout for those athletes who were subjected to stress more than those who were subjected to aerobic bouts.

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 exercise bouts resulted in an increase in CD34+ (SC) (Table 3). On the other hand, CD34+ (SC) tended to increase following anaerobic exercise bouts.

The number of circulating EPSs likely represents a balance between liberating of EPCs from the bone marrow and incorporation at the level of the vessel or differentiation. Laufs *et al.* (2005) demonstrated that CD34+/KDr+ increased after 30 minutes of high intensity running in healthy participants, but returned to resting levels within 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 had high EPC counts in the peripheral blood (Rehman *et al.,* 2004 And Laufs *et al.,* 2005).

Giuseffe *et al.* (2005) reported an increase in CD34+ stem cells and reticulocytes after supramaximal exercise, and they added that it was unlikely that this increase depended upon changes in blood or plasma volume, because these were much smaller than the changes in cell counts. either been incorporated for endothelial repair, neovascularizaiton or have undergone differentiation.

Ewa and Pawet, (2007) reported that a decrease in the blood supply to a body 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 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 the plasma of 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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