Relationship between endothelial progenitor cells and vascular endothelial growth factor and its variation with exercise

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RELATIONSHIP BETWEEN ENDOTHELIAL PROGENITOR CELLS AND VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS VARIATION WITH EXERCISE.

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Short Title:

ENDOTHELIAL PROGENITOR CELLS, VEGF AND EXERCISE IN CORONARY ARTERY DISEASE

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Abstract

Background: The aim of our study was to evaluate the effect of programmed physical activity and a single exercise test on the number of $CD309^+$ circulating endothelial progenitor cell (EPC) and their relation to the variation in plasma levels of VEGF in chronic coronary patients.

Methods: 21 patients <75 years with chronic stable coronary artery disease were included. All patients underwent exercise myocardial perfusion SPECT. Then, participants were divided into two groups: one group (11 patients) underwent cardiac rehabilitation program and the other (10 patients) continued with the standard treatment. Blood samples were obtained at baseline, 30 minutes after exercise ended and at one and three months during follow-up.

Results: VEGF values decreased significantly after exercise SPECT test. After one month, there was a significant increase in VEGF levels compared to those measured immediately after exercise. All patients showed a decrease in the values of EPC at 1 and 3-month follow-up. There was an inverse and statistically significant relation between change of EPC and VEGF between the baseline and 1 month.

Conclusions: The increase of VEGF at 1-month, with respect to baseline values correlated with decreased levels of EPC. This association was independent of the onset of ischemia in the perfusion study.

Abstract word count: 202

Keywords: Coronary artery disease, exercise, VEGF levels, progenitor cells

Introduction

The programmed exercise is a very important tool complementary to medical treatment and myocardial revascularization in patients with chronic coronary disease.

One of the proposed mechanisms of action, is that physical activity promotes angiogenesis by increasing the levels and/or half-life of vascular endothelial growth factor (VEGF). Furthermore ischemia is a major stimuli for VEGF production and for local enhancements of its effects.[1, 2]

It has also been established that endothelial progenitor cells (EPC) characterized by coexpression of Sca-1 receptor 2 of VEGF or VEGF-2 or KDR [3] improve angiogenesis, promote vascular repair, improve endothelial function, inhibit atherosclerosis and increase the ventricular function after myocardial infarction.

Physical training appear to be the most effective intervention to stimulate EPC [4] in both healthy subjects and patients with coronary disease. [5-7]

Exercise increases the production and the number of EPCs in patients with coronary disease and this increase would be time-dependent after a single episode of exercise-induced ischemia. [7-9]

However, extreme exercise such as marathon running, seems to decrease the number of circulating EPC defined with $CD34^+$ or $CD133^+$ after the race and even after several days[10, 11], while the total number of EPC remains unchanged. [11]

The increase in the number and migratory activity generated by exercise could be mediated by an upregulation of NO and VEGF as much as by a reduction in apoptosis of EPC.[6]

It has been shown that VEGF is one of the most potent stimuli for the release of EPC. [12, 13] VEGF would activate metalloproteinase-9 (MMP-9), which stimulates stem cells to migrate from a quiescent bone marrow niche to the vascular site. [14] The peak of the increase of VEGF clearly precedes the rising levels of EPC. [15] Furthermore, the antigen CD309 is a high affinity receptor for VEGF and plays an important role in hematopoiesis and is also involved in angiogenesis, in embryogenesis and in the context of homeostatic and pathological events. CD309-VEGFR-2 has been identified in subsets of hematopoietic stem cells, EPC and mature endothelial cells.

Therefore, the goal of the study was to evaluate in chronic coronary patients, the effect of acute exercise (as the stress test) on EPC and VEGF and its relationships according to the presence of ischemia. A second goal was to study in these patients, the effects of chronic exercise (as rehabilitation program) on the same parameters.

Methods

This study included 21 patients, < 75 years, with chronic stable coronary artery disease documented by coronary angiography, previous infarction or perfusion tests positive for ischemia performed more than 6 months ago. They should not have participated in groups of programmed physical activity within the last 3 months.

Patients unable to perform physical activity, or with extracardiac conditions affecting survival, associated cardiomyopathies or valvular heart disease, severe ventricular arrhythmia, heart failure, uncontrolled hypertension, FC III-IV angina despite treatment or a positive high-risk exercise stress test were excluded. All patients gave written informed consent to the study protocol, which was approved by the ethical committee.

After baseline evaluation, all the patients underwent exercise stress myocardial perfusion SPECT. According to the test results, two groups were defined:

- Patients with normal perfusion test.
- Patients with positive perfusion test defined by the presence of exercise-induced myocardial ischemia.

Then, the patients were randomly assigned to two groups:

- Patients not undergoing programmed physical activity (control group) (10 patients).
- Patients undergoing rehabilitation program with three exercise bouts weekly over 12 weeks (11 patients).

Aggravation of symptoms, manifestation of events or development of physical disability to continue with the exercise were considered causes of exclusion during follow-up.

Determination of VEGF

Blood samples were obtained at baseline, 30 minutes after exercise, and at one and three months during follow-up by vein puncture in tubes containing ethylenediaminetetraacetic acid tripotassium salt (K3EDTA) and stored at -70°.

Determination of VEGF was performed using Quantikine Human VEGF Immunoassay (R&D, catalog # DVE00) that employs the quantitative sandwich enzyme immunoassay technique with a monoclonal antibody specific for VEGF. This technique allows determination of VEGF in a range from 15.6 to 1000 pg/mL. The intra-assay precision and inter-assay precision are 5% and 8%, respectively.

Determination of EPC

Venous blood samples were collected in tubes containing K3EDTA and preserved at room temperature until they were processed.

Samples were incubated with lysis solution at room temperature for 20 minutes (2 ml of blood with anticoagulant in 14 ml of lysis liquid), then they were centrifuged (300xg, 10 minutes), the supernatant was discarded, and the cell button was resuspended in PBS. This procedure was repeated twice in order to obtain a purified erythrocyte-free population of cells.

Sixty µl of cells were incubated with 20 µl of FcR at room temperature during 15 minutes. Then they were washed and resuspended in 4 aliquots of 100 µl each:

Aliquot 1: Mouse IgG1-FITC + Mouse IgG1-PE (as negative controls)

Aliquot 2: CD45-FITC + Mouse IgG1-PE

Aliquot 3: CD45-FITC + CD34-PE

Aliquot 4: CD34-PE + CD309-PE

All aliquots were incubated in the dark during 40 minutes, and afterwards they were diluted with 450 µl of Isoflow and passed through the cytometer.

The samples were acquired on FACSCan -Becton Dickinson equipped with an Argon laser operated at 488 nm and analyzed by flow cytometer with CellQuest software.

CD45-FITC/anti-IgG-PE was used in aliquot 2 to compensate and delimit the $CD45^+$ region used for further acquisitions. So red cells, platelets and cells debris were excluded from analysis.

Region 1 (R1) was obtained from double positivity quadrant ($CD34^+/CD45^+$), we took into account cells that also expressed CD34+ from all mononuclear cells ($CD45^+$) (aliquot 3).

Samples of aliquot 4 were acquired to inform the expression of double positivity, and analyzed $CD34^+/CD309^+$, using R1 pre-labeled with aliquot 3. CD34 cells that coexpress CD309 support the existence of ECPs.

The amount of ECP is expressed as mean ± standard error (SEM) /100000 events. The events represent the passage of cells by laser equipment.

Myocardial perfusion test

Stress-rest SPECT imaging with ^{99m}Tc-sestamibi was performed within the same day, starting either with rest or stress image acquisition.

A cycle ergometer was used for exercise stress test following the Bruce protocol under vital signs control and electrocardiographic monitoring. The test was stopped according to conventional criteria.

The radioisotope was injected at rest and at peak exercise. Images were obtained 30 and 60 minutes after each injection of the radioisotope, using a step and shoot method in 64 projections. Rest and stress images were acquired in the short-axis view and horizontal and vertical long-axis views. The images were interpreted using a 17-segment heart model. A grading scale of 0 to 4 was used to score perfusion in each segment, where 0 = normal perfusion, 1 = mild hypoperfusion, 2 = moderate hypoperfusion, 3 = severe hypoperfusion, and 4 = absence of perfusion. The sum of the perfusion defects at rest and during stress constituted the summed rest score (SRS) and the summed stress scores (SSS). The summed difference score (SDS) or ischemia was calculated by subtracting the SRS from the SSS. A SDS ≥ 2 was considered positive for ischemia.

Cardiac rehabilitation program

Physical activity was performed twice a week in a rehabilitation center and once a week at the patient's home. During this period the patients continued with their regular anti-ischemic medication.

Rehabilitation plan consisted in calisthenics, biking with and without workload, gym or recreational activity, and long walks especially designed for each patient.

Statistical analysis

Categorical variables are presented as frequencies with the corresponding percentage. Continuous variables are expressed as mean ± standard error of

the mean (SEM) and median (interquartile range) according to the data distribution. The distribution of quantitative variables was established by analyzing the asymmetry (skewness) and kurtosis, and the Shapiro-Wilk test.

Discrete variables were analyzed with the chi-square test or Fisher's exact test, as applicable.

For continuous variables, t test for two independent samples or Kruskal-Wallis test after verification of the homogeneity of variance with Bartlett's test. A paired t test was used to compare intragroup differences of continuous variables.

For the analysis of the relationship between VEGF and EPC values and its variations, the Pearson correlation coefficient was used. A p value <0.05 was considered significant.

Results

Twenty-one patients were included; mean age was 62.5 ± 1.7 years. Seventeen patients were male (81.0%). Baseline characteristics are summarized in Table 1.

Ischemia was present in 9 patients with median SDS of 4 (2-6). There were no differences in the result of the exercise test according to the presence of ischemia in SPECT. The maximal heart rate during stress test was 120,3 ± 5.4 bpm in patients with ischemia and 119.3 ± 4.8 bpm in patients without ischemia (p=0.89). The maximal double-product was 20373 ± 1002 and 20422 ± 1487 respectively (p=0.89).

10 patients were assigned to programmed physical activity (6 had ischemia in SPECT) and 11 patients continued with standard treatment (control group) (4 with ischemia in SPECT).

Results of the measurement of EPC are summarized in Table 2. No differences between baseline and immediately after exercise were observed, either by the presence of ischemia in perfusion study or not.

All patients showed a decrease in the values of EPC at 1 and 3-month follow-up regardless of programmed physical activity.

The results of VEGF levels are summarized on Table 3. VEGF values decreased significantly after exercise SPECT test (49.49 ± 6.06 pg/mL vs. 31.83 ± 5.62 pg/mL; p=0.021). After one month, there was a significant increase in VEGF levels compared to those measured immediately after exercise (70.90 ± 14.44 pg/mL; p=0.007) and a non-significant trend compared to baseline values.

At 3 months, VEGF values presented a non-significant increase compared to baseline values (p = 0.12) and were similar to the levels measured after one month.

Based on the presence of ischemia in myocardial perfusion, the results were similar to those of the general population. There was no relationship between changes in the VEGF and the degree of ischemia observed in SPECT.

No statistically significant differences were observed in the follow-up at 1 and 3 months in patients who underwent programmed physical exercises.

No differences according to the presence of ischemia in patients in the control group and rehabilitation group were observed (Table 3).

There was a moderate positive correlation between baseline EPC $CD309^{+}$ and VEGF (r = 0.44; p = 0.047). This relationship was more significant in patients without ischemia in perfusion study (Table 4).

Considering the variation of EPC and VEGF with the "acute" (basal - post-effort) exercise globally, we observed that there was no relationship between them.

By correlating the delta of EPC and VEGF between the baseline sample and values at 1 month, it was inverse and statistically significant ($\Delta CD309^{+}$ / ΔVEGF: r = -0.57; p = 0.007) (figure 1).

A significant correlation was also observed in the variation of the values of EPC and VEGF between pos-exercise test and 1-month ($\Delta CD309^{+}$ / ΔVEGF: r = -0.59; p = 0.005).

These results were similar in both patients with and without ischemia, and in the control group. Patients in the rehabilitation group showed a non-significant trend (Table 4).

An increase of EPC values was observed at 3-month follow-up (compared with 1-month) without correlation with the change of VEGF.

Discussion

In this study we evaluated the effect of programmed physical activity and a single exercise test on the number of $CD309^+$ EPC and their relation to the variation in plasma levels of VEGF in chronic coronary patients. We did not observe a significant change in EPC levels with acute exercise. At 1-month there was a decrease in EPC in all patients (more marked in patients who performed programmed exercises). In the third month, EPC levels recovered to even exceeding baseline levels in the exercise group patients, although without statistical significance.

VEGF values decreased significantly with acute exercise. At 1-month follow-up there was a not statistically significant increase compared to baseline.

We did not observe a clear relationship between the change of VEGF and EPC between baseline and immediately after exercise test.

Although we detected a significant direct (but moderate) relationship between baseline VEGF and $CD309^+$ cells levels, it was mostly at the expense of patients with ischemia in perfusion study, suggesting that ischemia act as a trigger of both or have relation with baseline levels.

However, despite the decline of VEGF, there was no significant variation in levels of EPC with acute exercise.

Our results are different from those observed by Danzig et al [16] that evaluated the levels of VEGF and MMP-9 before and after exercise in patients with coronary disease and performed an exercise stress test. In this study, no significant differences in VEGF baseline levels compared to controls was

observed nor after exercise test, although there was a non-significant decrease in VEGF levels in patients with coronary artery disease).

The absence of variation of VEGF would be in accordance with the fact that exercise positively influences angiogenesis, which anyway is not directly determined by the VEGF elevation but by influencing the VEGF rate and the antiangiogenic protein, endostatin. [16]

Silva et al [17] showed in a systematic review that in healthy subjects, most studies evaluating a single session of maximum exercise observed no significant changes in plasma levels of VEGF.[8, 18, 19]

After a single session of exercise on a cycle-ergometer at 70% of the anaerobic threshold, the plasma levels of VEGF significantly increased only after 10 minutes of exercise with a significant correlation with the number of cells $CD133^{+}/KDR^{+}$.[20]

However, Adam et al [11] showed a decrease in plasma levels of VEGF after a marathon without correlation with the number of $CD34^{+}/VEGFR\text{-}2^{+}$ cells.

Higher plasma levels of VEGF were verified after periods of long / ultra long lasting exercises.[17] These would be associated with the presence of tissue hypoxia in these conditions, which favor the stimulus for EPC mobilization and endothelial repair.[17]

Increased VEGF levels would be a physiological adaptation of exercise and suggests a positive correlation between exercise intensity and the release of growth factors.[21] Thus, it would stimulate gene expression of EPC mobilizing molecules such as VEGF, which is in line with our observed increase of VEGF at 1 and 3 months.

The increase of VEGF at 1-month was correlated with decreased levels of EPC, which could mean higher incorporation of cells into tissues and therefore a lower circulating levels. This relationship was observed independently of the occurrence of ischemia in perfusion study.

At 3 months, values of EPC increased unrelated to the variation of VEGF so other mechanisms not studied in this protocol could be involved. Longer and/or more intense periods of training may be necessary to spell them out.

The discrepancies observed with previous studies may be due to different cell types studied, the period of time between exercise and blood collection, and the intensity and duration of exercises, both in "acute" exercise and "chronic" exercise.

The mobilization of progenitor cells, which is NO dependent,[22] could be improved by higher metabolic stress. However, this effect is relatively short and is not apparent after 48 hours post-exercise. Likewise, obtaining blood samples 24-48 hours after completing the rehabilitation program sessions might have influenced our results.

A limitation could be that most patients with chronic coronary artery disease who meet the inclusion criteria were men but the gender distribution is, in the population of our hospital, similar to that included in the study. Mobilization of progenitor cells could also be depending on exercise intensity suggesting a physiological stimulus to improve oxygen delivery via increased capillary network in the exercise above anaerobic threshold. In addition there are several cell types isolated from bone marrow, and peripheral stem cells resident in

tissues, which may have the potential to differentiate into endothelial blood cells, so that this variation may also contribute to the discrepancy in the results.

Conclusion

The increase of VEGF at 1-month, with respect to baseline values correlated with decreased levels of EPC, which could mean higher incorporation of cells into tissues and therefore a lower level of circulating cells. This association was independent of the onset of ischemia in the perfusion study. No relationship with chronic exercise (as the rehabilitation program) was observed.

Table 1. Clinical Characteristics of the Study Patients

Variables	Study Population		Control Group		Exercise Group		p
n	21		11		10		
Age (years) (mean ± SEM)	62.5 ± 1.7		65.4 ± 1.6		59.5 ± 2.8		0.08
	n	**%**	**n**	**%**	**n**	**%**	
Male gender	17	81.0	10	90.9	7	70.0	0.25
Coronary Risk Factors							
Hypertension	17	81.0	10	90.9	7	70.0	0.25
Dyslipidemia	14	66.7	6	54.4	8	80.0	0.22
Diabetes mellitus	2	9.5	2	18.2	0		0.26
Current smoker	2	9.5	1	9.1	1	10.0	0.74
Ex-smoker	10	47.6	6	54.4	4	40.0	0.41
Obesity	8	38.1	4	36.4	4	40.0	0.61
Coronary History							
Chronic stable angina	16	76.2	8	72.7	8	80.0	0.55
FC I	5	31.3	2	25.0	3	37.5	
FC II	11	68.8	6	75.0	5	62.5	0.50
Myocardial infarction	8	38.1	5	45.5	3	30.0	0.39
Angioplasty	7	33.3	3	27.3	4	40.0	0.44
CABG	2	9.5	1	9.1	1	10	0.74
Medical treatment							
Aspirin	20	95.2	10	90.9	10	100	0.52
Beta-blockers	20	95.2	10	90.9	10	100	0.52

Nitrates	7	33.3	4	36.4	3	30.0	0.56
Calcium-blockers	3	14.3	2	18.2	1	10.0	0.54
Converting Enzyme Inhibitors / AT II Blockers	13	61.9	6	54.5	7	70.0	0.39
Statins	17	81	9	81.8	8	80.0	0.67

Table 2. Measurement of endothelial progenitor cells (CD309+) in the study population and in the subgroups according to presence or absence of ischemia in perfusion study and the realization of programmed exercises.

	Baseline	After exercise test	Month 1	Month 3
Mean	0.166	0.149	0.044 *	0.091 ** ***
SEM	0.030	0.025	0.004	0.008
		Without Ischemia (n=12)		
Mean	0.254	0.132	0.039	0.088
SEM	0.025	0.020	0.003	0.009
		Ischemia (n=9)		
Mean	0.206	0.184	0.035	0.102
SEM	0.071	0.059	0.004	0.017
		Control Group (n=11)		
Mean	0.208		0.041	0.088
SEM	0.060		0.003	0.013
		Exercise Group (n=10)		
Mean	0.141		0.033	0.101
SEM	0.022		0.004	0.037

SEM, standard error of mean.

* P<0.01 vs Baseline; ** p<0.001 vs Month 1; *** p=0.006 vs Baseline

Table 3. VEGF levels in the study population and in the subgroups according to presence or absence of ischemia in perfusion study and the realization of programmed exercises.

	Baseline	After exercise test	Month 1	Month 3
Mean	49.59	31.83 *	70.90 ** ***	67.48
SEM	6.06	5.62	14.44	13.31
		Without Ischemia (n=12)		
Mean	46.69	34.29 *	78.71 ****	63.25
SEM	6.78	9.60	22.07	17.14
		Ischemia (n=9)		
Mean	53.44	28.56 *****	60.50 ******	73.11
SEM	11.26	3.65	17.35	22.11
	Basal		Mes 1	Mes 3
		Control Group (n=11)		
Mean	45.25		82.41	60.68 **
SEM	9.24		26.68	17.87
		Exercise Group (n=10)		
Mean	54.35		58.25	74.95
SEM	7.89		8.37	20.62

SEM, standar error of mean

* p=0.021 vs. Baseline; ** p=0.007 vs. After exercise test; *** p=0.10 vs. Baseline.

**** p=0.05 vs. After exercise test; ***** p=0.057 vs. Baseline; ****** p=0.07 vs. After exercise test.

Table 4. Correlation between EPC ($CD309^{+}$) and VEGF.

	Global		Ischemia		Without Isquemia		Exercise		Control	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Baseline	0.44	**0.047**	0.34	0.37	0.68	**0.01**	0.10	0.79	0.47	0.12
After exercise test	0.15	0.52	0.60	0.08	0.08	0.81				
Month 1	0.14	0.55	-0.23	0.95	0.21	9.52	0.56	0.12	0.01	0.97
Month 3	0.13	0.58	-0.23	0.95	-0.05	0.23	-0.03	0.93	0.24	0.45
ΔEPC / ΔVEGF Baseline-Month 1	-0.57	**0.007**	-0.81	**0.007**	-0.77	**0.003**	-0.50	0.16	**-0.58**	**0.04**
ΔEPC / ΔVEGF After exercise test-Month	-0.59	**0.005**	-0.88	**0.001**	-0.69	**0.018**				

1										
ΔEPC / ΔVEGF Month 1-Month 3	-0.29	0.19	-0.39	0.29	-0.42	0.17	0.17	0.66	-0.45	0.13

Figure Legend

Figure 1. Correlatiof the delta of EPC and delta of VEGF between the baseline sample and values at 1 month.

Declaration of Conflicting Interest

None declared

References

[1] Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation. 2001;103:2776-9.

[2] Sandri M, Adams V, Gielen S, Linke A, Lenk K, Krankel N, et al. Effects of exercise and ischemia on mobilization and functional activation of blood-derived progenitor cells in patients with ischemic syndromes: results of 3 randomized studies. Circulation. 2005;111:3391-9.

[3] Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. Circ Res. 2004;95:343-53.

[4] Fadini GP, Agostini C, Sartore S, Avogaro A. Endothelial progenitor cells in the natural history of atherosclerosis. Atherosclerosis. 2007;194:46-54.

[5] Steiner S, Niessner A, Ziegler S, Richter B, Seidinger D, Pleiner J, et al. Endurance training increases the number of endothelial progenitor cells in patients with cardiovascular risk and coronary artery disease. Atherosclerosis. 2005;181:305-10.

[6] Laufs U, Werner N, Link A, Endres M, Wassmann S, Jurgens K, et al. Physical training increases endothelial progenitor cells, inhibits neointima formation, and enhances angiogenesis. Circulation. 2004;109:220-6.

[7] Adams V, Lenk K, Linke A, Lenz D, Erbs S, Sandri M, et al. Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. Arterioscler Thromb Vasc Biol. 2004;24:684-90.

[8] Van Craenenbroeck EM, Vrints CJ, Haine SE, Vermeulen K, Goovaerts I, Van Tendeloo VF, et al. A maximal exercise bout increases the number of circulating CD34+/KDR+ endothelial progenitor cells in healthy subjects. Relation with lipid profile. J Appl Physiol. 2008;104:1006-13.

[9] Rehman J, Li J, Parvathaneni L, Karlsson G, Panchal VR, Temm CJ, et al. Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. J Am Coll Cardiol. 2004;43:2314-8.

[10] Bonsignore MR, Morici G, Santoro A, Pagano M, Cascio L, Bonanno A, et al. Circulating hematopoietic progenitor cells in runners. J Appl Physiol. 2002;93:1691-7.

[11] Adams V, Linke A, Breuckmann F, Leineweber K, Erbs S, Krankel N, et al. Circulating progenitor cells decrease immediately after marathon race in advanced-age marathon runners. Eur J Cardiovasc Prev Rehabil. 2008;15:602-7.

[12] Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, et al. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J. 1999;18:3964-72.

[13] Kalka C, Masuda H, Takahashi T, Gordon R, Tepper O, Gravereaux E, et al. Vascular endothelial growth factor(165) gene transfer augments circulating endothelial progenitor cells in human subjects. Circ Res. 2000;86:1198-202.

[14] Heissig B, Werb Z, Rafii S, Hattori K. Role of c-kit/Kit ligand signaling in regulating vasculogenesis. Thromb Haemost. 2003;90:570-6.

[15] Khakoo AY, Finkel T. Endothelial progenitor cells. Annu Rev Med. 2005;56:79-101.

[16] Danzig V, Mikova B, Kuchynka P, Benakova H, Zima T, Kittnar O, et al. Levels of circulating biomarkers at rest and after exercise in coronary artery disease patients. Physiol Res. 2010;59:385-92.

[17] Silva JF, Rocha NG, Nobrega AC. Mobilization of endothelial progenitor cells with exercise in healthy individuals: a systematic review. Arq Bras Cardiol. 2012;98:182-91.

[18] Thijssen DH, Vos JB, Verseyden C, van Zonneveld AJ, Smits P, Sweep FC, et al. Haematopoietic stem cells and endothelial progenitor cells in healthy men: effect of aging and training. Aging Cell. 2006;5:495-503.

[19] Yang Z, Wang JM, Chen L, Luo CF, Tang AL, Tao J. Acute exercise-induced nitric oxide production contributes to upregulation of circulating endothelial progenitor cells in healthy subjects. J Hum Hypertens. 2007;21:452-60.

[20] Mobius-Winkler S, Hilberg T, Menzel K, Golla E, Burman A, Schuler G, et al. Time-dependent mobilization of circulating progenitor cells during strenuous exercise in healthy individuals. J Appl Physiol. 2009;107:1943-50.

[21] Bonsignore MR, Morici G, Riccioni R, Huertas A, Petrucci E, Veca M, et al. Hemopoietic and angiogenetic progenitors in healthy athletes: different responses to endurance and maximal exercise. J Appl Physiol. 2010;109:60-7.

[22] Cubbon RM, Murgatroyd SR, Ferguson C, Bowen TS, Rakobowchuk M, Baliga V, et al. Human exercise-induced circulating progenitor cell mobilization

is nitric oxide-dependent and is blunted in South Asian men. Arterioscler Thromb Vasc Biol. 2010;30:878-84.

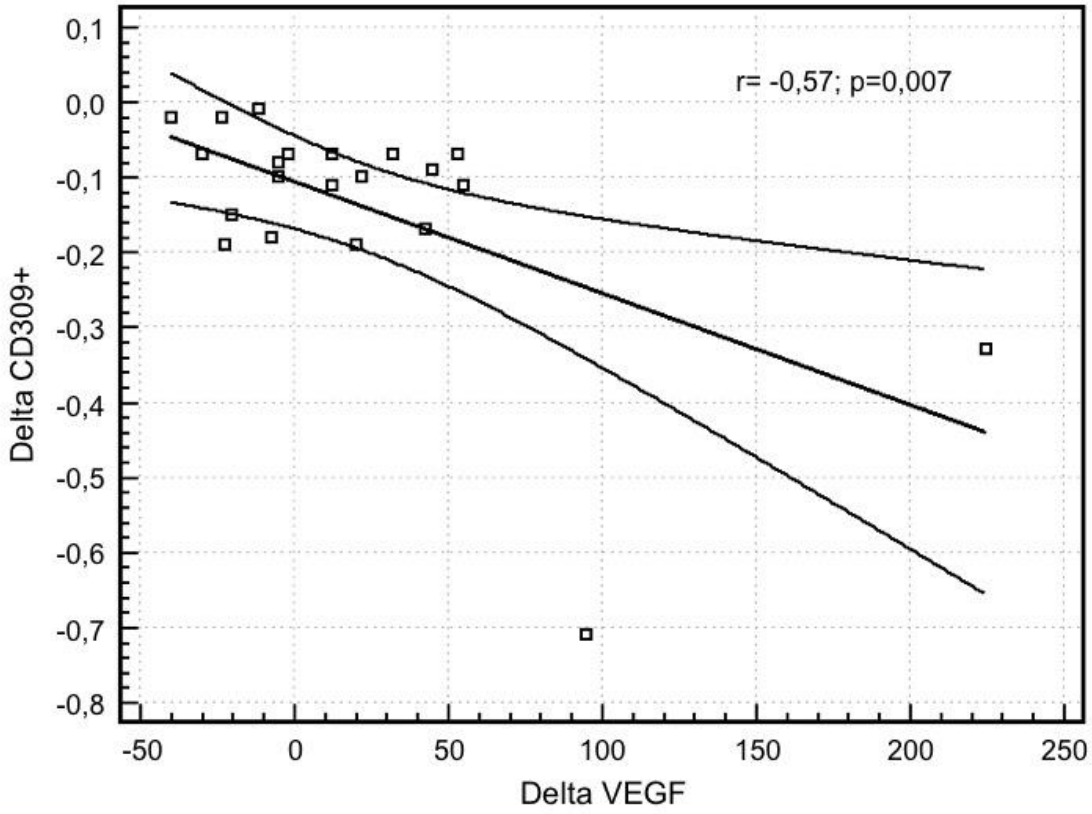

Figure 1

Highlights

1. VEGF values decrease after acute exercise and significantly increase after 1 month
2. CD309 endothelial cells decrease after 1 and 3 months independently of exercise
3. An inverse relation between change of EPC and VEGF at 1 month was observed
4. These associations were independent of the presence of ischemia or type of exercise