Clinical Hemorheology and Microcirculation 80 (2022) 281–289
DOI 10.3233/CH-211229
IOS Press

Responses of platelet CD markers and indices to resistance exercise with and without blood flow restriction in patients with type 2 diabetes

Elahe Malekyian Fini[a], Morteza Salimian[b] and Sajad Ahmadizad[a,*]
[a]*Department of Biological Sciences in Sport, Faculty of Sport Sciences and Health, Shahid Beheshti University, Tehran, Iran*
[b]*Platelet Research Laboratory, Kashan University of Medical Sciences, Kashan, Iran*

Abstract.
BACKGROUND: Diabetes mellitus is a common disorder with the risk of vascular injury.
OBJECTIVE: The aim of this study was to compare the effects of low-intensity resistance exercise with blood flow restriction versus high-intensity resistance exercise on platelet CD markers and indices in patients with type 2 diabetes.
METHODS: Fifteen female patients with type 2 diabetes (Mean ± SD; age, 47.6 ± 7.2 yrs) randomly completed two resistance exercise at an intensity corresponding to 20% and 80% of one-repetition maximum (1-RM), with and without blood flow restriction (RE_{BFR} and RE), respectively. We measured markers of platelet activation (P-selectin, GpIIb/IIIa, and CD42) and platelet indices before and immediately after exercise, and after 30 min recovery.
RESULTS: Platelet count (PLT) and plateletcrit (PCT) increased in response to RE_{BFR} more than the RE ($p < 0.05$), though, no significant differences in PDW and MPV were observed ($p < 0.05$). Although P-selectin (CD62P), CD61, CD41, and CD42 were reduced following resistance exercise in both trials, these reductions were non-significant ($p < 0.05$). Besides, no significant between-group differences were found for platelet CD markers ($p < 0.05$).
CONCLUSIONS: It is concluded that RE_{BFR} induces thrombocytosis, but responses of platelet CD markers in patients with type 2 diabetes are similar following low-intensity RE_{BFR} and high-intensity RE.

Keywords: Strength training, kaatsu, type 2 diabetes, thrombosis, platelet activation, P-selectin

1. Introduction

Type 2 diabetes is a multifactorial disease that is caused by a combination of environmental and genetic factors [1]. Diabetes is associated with altered platelet function, where platelet adhesion and aggregation are increased by hyperglycemia and lead to thrombus formation [1, 2]. The increased risk of thrombosis in patients with type 2 diabetes and metabolic syndrome is due to increases in platelet aggregation, fibrinogen, coagulation factors, and fibrinolysis [3–6]. In patients with type 2 diabetes, insulin resistance, hyperglycemia, and increased glycolysis are associated with increased platelet sensitivity to platelet aggregation receptors [7], whereas, normal glucose concentration attenuates this process [8]. In addition, diabetic ketoacidosis patients have an increased risk of arterial and venous

*Corresponding author: Sajad Ahmadizad, Department of Biological Sciences in Sport, Faculty of Sport Sciences and Health, Shahid Beheshti University, Tehran, Iran. E-mail: s_ahmadizad@sbu.ac.ir.

ISSN 1386-0291/$35.00

thromboembolism and that clots formed during ketoacidosis with high glucose levels are very dense and thrombogenic [9].

Regular exercise improves insulin sensitivity and reduces blood glucose levels in patients with type 2 diabetes [10, 11]. Although regular exercise reduces the major risk of thrombotic and cardiovascular events [12], the risk of early heart attacks increases dramatically during acute vigorous exercise [13]. Resistance exercise increases platelet activity and function [14] in an intensity-related manner [15].

Although resistance exercise at an intensity greater than 70% of maximum strength increases muscle size and strength in healthy individuals [16], patients often cannot complete resistance exercise at the desired intensities. However, low-intensity resistance exercise with blood flow restriction (BFR) is useful in physical rehabilitation programs to achieve a similar degree of muscle mass and strength compared to high-intensity resistance exercise [17]. During BFR, a low-intensity resistive load in combination with an external inflation cuff is used to partially occlude the underlying vasculature and likely induces muscle hypoxia and metabolic stress [18].

Efficacy and patient safety associated with any rehabilitation program is a necessary concern for health professionals [19]. Low-intensity resistance exercise with BFR induces lower physical stress compared to traditional resistance exercise, which would further enhance safety. The concern regarding the potential of BFR to increase the risk of thrombosis or thrombocytosis remains for some clinicians. Although BFR causes a temporary reduction in blood flow in the exercising limb, it occurs for a relatively short period, and blood flow is back to normal following cuff deflation.

Since BFR is presently a novel form of rehabilitation exercise and its safety remains an issue, health, and sports medicine professionals do not include it in rehabilitation programs. Although, BFR with acute resistance exercise does not activate the coagulation system in healthy subjects [20], the safety and acute effects of low-intensity resistance exercise with BFR on thrombosis particularly in patients with diabetes have not yet been addressed. Therefore, the purpose of the present study was to determine the effect of an acute bout of low-intensity resistance exercise with BFR compared to traditional high-intensity resistance exercise on the platelet indices and markers of platelet activation and function.

2. Materials and methods

2.1. Subjects

Fifteen women with type 2 diabetes (Mean $\pm$ SD; age, 47.6 ± 7.2 yrs; height, 156.8 ± 6.9 cm; weight, 69.7 ± 13.8 kg; BMI, 28.43 ± 5.39 kg/m^2; WHR, 0.86 ± 0.06) with no history of regular exercise voluntarily participated in the study. Their mean fasting blood sugar was 172.5 ± 18.8 mg/dL. Participants were screened and consulted by a cardiologist for any heart condition including ischemia, angina pectoris, arrhythmia, heart failure, and abnormal ECG. Participants received no more than two medicines at the time of the study and did not receive insulin, antiplatelet drugs such as aspirin, NSAID, and Clopidogrel. Participants who had bone or joint problems (lower back and knee), muscle injury, any other condition that stopped them from doing exercise, a history of specific infectious and immune disorders, smoking, and alcohol consumption were excluded. Participants completed a Medical History Questionnaire as well as an informed consent form after explaining the study protocols and having understood the procedures. This study is carried out conforming to the guidelines for the use of human subjects in research as outlined in the current Declaration of Helsinki and was approved by the University Ethical Board (IR.KAUMS.REC.1399.035) and Registry of Clinical Trials (IRCT20130901014540N2).

2.2. Experimental design

Two preliminary sessions were designed to familiarize the participants with the correct techniques for resistance exercises and to determine the maximal strength by using a one-repetition maximum test (1-RM).

After familiarization with the exercise techniques, participants' unilateral 1-RM for knee flexion and extension was determined on a weight-training machine by using Brzycki's method [21]. This method is a submaximal estimation method and is preferred because it is safer and quicker than the other methods. In this method, 1-RM is calculated based on the number of repetitions performed and the amount of weight used. In this session, we measured participants' weight (kg) and height (cm) to the nearest 0.1 kg and 0.1 cm, respectively, on an automatic electronic scale (Seca - Germany).

After 1-RM determination, participants attended the laboratory in two different sessions with one week intervening and performed unilateral knee flexion and extension exercises either with or without BFR in a randomized, crossover design. Participants were instructed to refrain from exercise for 48 h before reporting to the laboratory for testing. In addition, they were required to eat their dinner at 08:00 p.m., do not drink any fluid after midnight, and attend the laboratory in a fasting state. To avoid the effects of time of day, all experimental sessions were performed at the same time of day (0900–1100 h).

In each trial, after changing the attire, participants rested in the sitting position for 20 min where the first fasting blood sample was taken. Thereafter, they had 5 min general warm-up and 2, 3 min static and dynamic stretching exercises for thigh and leg muscles. After the general warm-up and prior to the main exercise protocol, a specific warm-up including knee flexion and extension (2 sets of 8 repetitions) with an unloaded machine and a light load was performed. The resistance exercise protocol consisted of 3 sets of 6 repetitions at 80% of 1-RM for knee flexion and extension with the dominant leg. Two minutes of rest was allowed between sets and exercises. A second venous blood sample was taken immediately after exercise. Participants were then rested on the chair for 30 min and at the end of this period (recovery), the third blood sample was taken. At the end of each set, we recorded heart rate and blood pressure. The second exercise trial included performing 3 sets of 15 repetitions at 20% of 1-RM for knee flexion and extension exercises with BFR. For the BFR condition, a lower extremity pressure cuff (AS-027, ABN Company, Indonesia) with a range of 406 to 660 mm was placed around the most proximal portion of the exercised leg and inflated to a pressure of 150 mmHg. In this session, 30 s rest was given between sets and exercises, and all measurements were made similar to the exercise trial without BFR.

2.3. Blood sampling and laboratory methods

Three venous blood samples (21 ml) from a brachial vein were drawn in a sitting position before exercise (after 20 min rest), immediately after exercise, and after 30 min recovery. Two milliliters of blood was transferred into the tubes containing ethylenediamine tetraacetic acid (k2EDTA) and slowly mixed. Anicoagulated blood was used for measuring hematocrit (Hct) and hemoglobin (Hb), platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW) by Coulter Counter (Sysmex, XE-2100L, Japan). In addition, 5 ml of blood was dispensed into tubes containing citrate theophylline adenosine dipyridamole (CTAD) anticoagulant and slowly mixed. Flow cytometry (Partec Cyflow, Germany) technique was used to measure P-selectin, GpIIb/IIIa (CD41, CD61), and CD42. We measured changes in platelet activity and response to agonists by using the direct immunofluorescence techniques. The following fluorescein-isothiocyanate (FITC) and phycoerythrin (PE)-conjugated monoclonal antibodies (MAbs) including anti-CD41-PE (clone P2), anti-CD42b-PE (clone SZ2), and anti-CD62P-FITC (Clone CLB-Thromb/6) were all purchased from Coulter-Immunotech Diagnostics (Krefeld, Germany). Plasma volume changes (PV) were calculated

by using Hb and Hct values before and after exercise and according to the equations described by Dill and Costill [22].

2.4. Statistical analysis

Data were analyzed using SPSS version 22 for Windows (IBM, Chicago, IL). The distributions of data were checked for normality by using the Shapiro-Wilk test. We employed repeated measures of ANOVA (2 conditions × 3 times) to compare the responses of platelet indices and markers of platelet activation to resistance exercise with or without BFR. Paired *t*-test was used to compare the plasma volume changes in two trials. Differences between conditions were considered significant at $P < 0.05$ and data are presented as means ± SD.

3. Results

Statistical analysis of the data revealed a significant interaction for PLT ($F_{2,28} = 3.8$, $p = 0.03$). Platelet counts increased in response to RE_{BFR} more than the resistance exercise without BFR. Immediately after RE_{BFR} platelet count increased by 7.5% significantly ($P < 0.05$) and returned to baseline level after 30 min of recovery (Table 1), whereas, changes in PLT after resistance exercise without BFR (1.2% increase) were non-significant. Similarly, changes in PCT following RE_{BFR} were significantly ($F_{2,28} = 8.4$, $p = 0.001$) higher than the resistance exercise without BFR, and that *post-hoc* analyses showed significant differences in response to exercise, not recovery (Table 1). However, neither a significant interaction nor significant main effects of exercise ($p > 0.05$) were observed for MPV and PDW (Table 1). Plasma volume reduced by 4.7% and 2.4% following resistance exercise with and without BFR, respectively. Plasma volume changes in response to exercise and recovery were not significantly different between the two trials ($p > 0.05$).

The results of the study showed no significant difference between responses of P-selectin to resistance exercise with and without BFR ($p > 0.05$). In addition, no significant main effect of resistance exercise was detected for P-selectin (Fig. 1).

Mean values of the CD61 receptor at rest, immediately after RE_{BFR}, and at the end of the recovery period were 16.49 ± 1.07, 15.93 ± 1.00, and 16.01 ± 1.00 μg/mL, respectively (Fig. 2). Neither a significant main effect nor a significant session × time interaction was detected when CD61 values were compared between two trials. However, within-group comparisons showed a significant ($p = 0.02$) reduction in CD61 following resistance exercise, which remained at the same level during recovery (Fig. 2).

Table 1
Mean (±SD) values of platelet indices before and immediately after exercise, and after 30 min recovery with and without BFR

	RE_{BFR}			RE		
	Before	After	Recovery	Before	After	Recovery
PLT (1000/μL)	294 ± 13	317 ± 15*#	302 ± 14	317 ± 17	321 ± 16	297 ± 14
MPV (fl)	9.40 ± 0.19	9.40 ± 0.16	9.26 ± 0.18	9.40 ± 0.25	9.26 ± 0.20	9.33 ± 0.21
PCT (%)	0.213 ± 0.007	0.253 ± 0.008*#	0.217 ± 0.008	0.215 ± 0.010	0.237 ± 0.014	0.211 ± 0.010
PDW (%)	11.86 ± 0.40	11.93 ± 0.35	11.93 ± 0.35	11.60 ± 0.42	11.66 ± 0.38	11.73 ± 0.39

PLT, platelet count; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width. *Indicates a significant increase following exercise ($p < 0.05$) and # indicates between-group significant difference ($p < 0.05$).

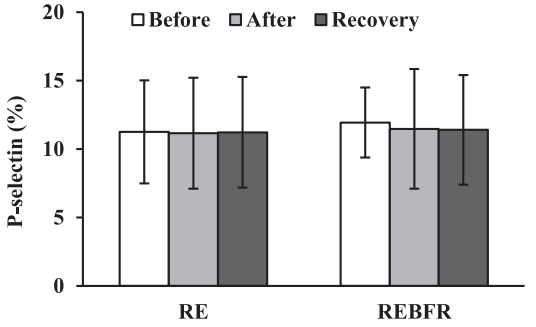

Fig. 1. Mean (±SD) values of P-selectin before (open bars) and immediately after exercise (grey bars), and after 30 min recovery (solid bars) with and without BFR.

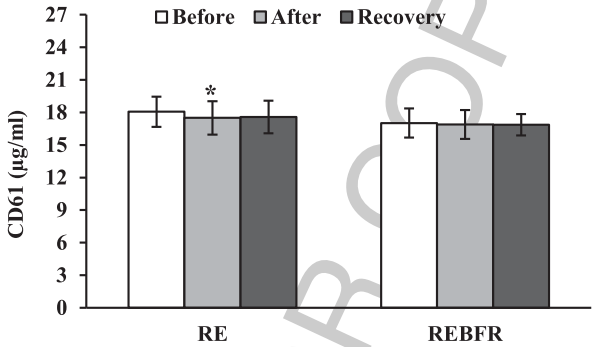

Fig. 2. Mean (±SD) values of CD61 before (open bars) and immediately after exercise (grey bars), and after 30 min recovery (solid bars) with and without BFR. Significant ($p < 0.05$) difference between pre- and post-exercise is denoted by*.

Mean values (±SD) of CD41 at rest, immediately after resistance exercise and recovery for BFR and without BFR trials were 14.53 ± 0.95, 14.25 ± 0.86, 14.30 ± 0.98 and 16.06 ± 1.32, 14.65 ± 1.02, 15.14 ± 0.98 μg/mL, respectively. The CD42 values (±SD) at rest, immediately after resistance exercise and recovery for BFR and without BFR trials were 12.63 ± 0.51 to 12.17 ± 0.31, 12.92 ± 0.42 and 14.28 ± 0.79, 13.44 ± 0.47, 14.57 ± 1.13 μg/mL, respectively. Statistical analyses revealed neither a significant main effect of exercise nor a significant session × time interactions for CD41 and CD42 ($P > 0.05$).

4. Discussion

Numerous studies have been conducted to determine the acute effect of different endurance and resistance exercise protocols on platelet variables [14, 23]. Previous studies have shown that vigorous endurance and resistance exercise are associated with thrombocytosis [14, 23], possibly due to increased platelet release from the spleen, bone marrow, and lungs [24]. We found increases in platelet count and plateletcrit (percent of blood platelets) following exercise, and that the changes following RE_{BFR} were higher than the resistance exercise alone. These findings confirm those of previous studies that demonstrated increases in PLT following different resistance exercise protocols [15] and increases in PLT under hypoxic conditions [25]. For example, PLT is increased following circuit resistance exercise and different intensities of traditional resistance exercise [15]. In our study, low-intensity resistance exercise combined with hypoxia resulted in higher increases in PLT. During vascular occlusion, the

obstructive muscles are affected by oxygen depletion during exercise [26] and it is assumed that anaerobic metabolism is more involved in the process of energy production during vascular occlusion [26]. Therefore, hypoxia-induced changes in blood pH levels following the release of H^+ ions during activity, and increases in shear stress and catecholamine (result in fresh release of platelets from marginal pools) might explain higher changes in PLT following exercise with BFR [26]. Although, we did not measure lactate concentration in the present study, higher changes in RE_{BFR} might be attributed to higher increases in lactate accumulation, which has been confirmed previously [27].

However, the amount of changes in PLT following resistance exercise in our study was less than the previous resistance exercise studies [14, 15] that might be due to low load or volume of exercise (sets × reps × amount of weight). In our study, two exercises including knee flexion and extension were employed in both trials. In our study, resistance exercise resulted in no significant changes in MPV and PDW that are consistent with findings of our earlier resistance exercise studies [14, 15].

Furthermore, we found that P-selectin (CD62P), a marker of platelet activation, did not increase immediately after exercise and no effect of BFR with exercise was found. These findings support those of Petridou et al. [28] who did not find any changes in the concentration of P-selectin after resistance exercise; whereas, the study by Smith et al. [29] reported a decreasing trend in P-selectin immediately after high-intensity eccentric resistance exercise. Increased hypoxia induces adenosine accumulation through intracellular ATP depletion, increased intracellular AMP, and activation of hypoxia-induced factor 1α (HIF-1α), which, stimulates CD39 and CD73 expression, endothelial cell membrane-bound enzymes that hydrolyze extracellular ATP to AMP [30, 31]. AMP is an anti-thrombotic and anti-platelet activator [32], which inhibits the P-selectin expression and GPllb/llla activity (integrin αllbβ3). On the other hand, hypoxic conditions lead to endothelial NO production [33], which in turn, activates the guanilyl cyclase signaling pathway in platelets. Therefore, these two hypoxia-dependent pathways might lead to the inhibition of platelet activation signaling pathways [34].

Both endurance and resistance exercise with restriction of blood flow increase eNOS expression and NO release, which stimulates cGMP activation [35]. This process results in the reduction of platelet adhesion and activation at the endothelial surface [36] and inhibition of growth factors activity released from cells and platelets [37].

Platelet microparticles (PMPs) are known as potential markers of thrombosis [38]. In line with the findings of the present study for CD41, CD42, CD61, and CD62p, Bittencourt et al. [39]. showed that the microparticles in professional runners did not increase after intense exercise. Similarly, some other studies [39, 40] found no changes in PMP and EMP (endothelial microparticles) levels even following high-intensity (100% power) exercise. Previous studies have demonstrated that P-selectin increases following exercise [41, 42] in an intensity related manner [41], and it has also been proven that changes in CD62P are highly dependent on shear stress [43]. In the present study, there were no differences between responses of blood pressure to two exercise protocols, and probably these two protocols have not resulted in high shear stress. During exercise, increases in cardiac output and blood flow to the exercising muscles elicit an increase in shear stress [44], which affects endothelial function [45]. On the other hand, high shear stress induces changes in the expression of platelet membrane glycoproteins such as PAC1, CD62P, GPIb/IX, and GPIIb/IIIa. Platelets are activated by high shear stress directly and independently [46], and the activation may be mediated by these glycoproteins. Therefore, the lack of changes in P-selectin and platelet microparticles in the present study might be attributed to the low volume/load of the resistance exercise in both trials that might have not been able to induce high shear stress.

It is recognized that there are limitations to the results of the present study. For example, only the acute effects of resistance exercise with blood flow restriction on platelet activation and function in patients with type 2 diabetes were examined. Further studies are needed to determine the chronic effects of RE_{BFR} on platelet activation and function. Another limitation of the present study was that the resistance

exercise volume was low and only two exercises (knee flexion and extension with the dominant leg) were involved. Probably lack of the resistance exercise effect on platelet activation markers might be attributed to the low load and volume of the resistance exercise. Therefore, further studies are warranted to shed some more lights on this by employing different combinations of resistance exercise volume and intensity.

In summary, the present study revealed that low-intensity RE with blood flow restriction results in higher thrombocytosis compared to traditional high-intensity resistance exercise in patients with type 2 diabetes, and that platelet activation did not occur with this exercise modality probably because of the load and volume of RE which was low in comparisons to previous studies. Since, low-intensity RE with BFR affected on platelet activation markers similar to high-intensity RE, and that it induced higher thrombocytosis, using light intensity RE with BFR is not suggested for patients with diabetes and finally prescribing the RE is not advised until further studies clarify this.

Acknowledgments

The authors would like to thank all the patients who participated in this study.

References

[1] Schafer A, Bauersachs J. Endothelial dysfunction, impaired endogenous platelet inhibition and platelet activation in diabetes and atherosclerosis. Curr Vasc Pharmacol. 2008;6(1):52-60. doi.org/10.2174/157016108783331295.

[2] Koscielny J, Latza R, Wolf S, Kiesewetter H, Jung F. Early rheological and microcirculatory changes in children with type I diabetes mellitus. Clin Hemorheol Microcirc. 1998;19(2):139-50. https://pubmed.ncbi.nlm.nih.gov/9849927/.

[3] Suslova TE, Sitozhevskii AV, Ogurkova ON, Kravchenko ES, Kologrivova IV, Anfinogenova Y, et al. Platelet hemostasis in patients with metabolic syndrome and type 2 diabetes mellitus: cGMP-and no-dependent mechanisms in the insulin-mediated platelet aggregation. Front Physiol. 2015;5:501. doi: 10.3389/fphys.2014.00501.

[4] Fox CS, Golden SH, Anderson C, Bray GA, Burke LE, de Boer IH, et al. American Heart Association Diabetes Committee of the Council on Lifestyle and Cardiometabolic Health, Council on Clinical Cardiology, Council on Cardiovascular and Stroke Nursing, Council on Cardiovascular Surgery and Anesthesia, Council on Quality of Care and Outcomes Research, and the American Diabetes Association. Update on Prevention of Cardiovascular Disease in Adults With Type 2 Diabetes Mellitus in Light of Recent Evidence: A Scientific Statement From the American Heart Association and the American Diabetes Association. Circulation. 2015;132(8):691-718. doi: 10.2337/dci15-0012.

[5] Wautier JL, Wautier MP. Cellular and Molecular Aspects of Blood Cell-Endothelium Interactions in Vascular Disorders. Int J Mol Sci. 2020;21(15):5315. doi: 10.3390/ijms21155315.

[6] Bach R, Jung F, Kohsiek I, Ozbek C, Spitzer S, Scheller B, Dyckmans J, Schieffer H. Factors affecting the restenosis rate after percutaneous transluminal coronary angioplasty. Thromb Res. 1994;74(Suppl 1):S55-67. doi: 10.1016/s0049-3848(10)80007-6.

[7] Gruzdeva OV, Kremeno SV, Suslova TE, Levashkina EA. Platelet-mediated hemostasis and parameters of carbohydrate metabolism in patients with combination of type 2 diabetes mellitus and arterial hypertension. Siberian Med J. 2008;4-2:28-30. doi: 10.3389/fphys.2014.00501.

[8] Dandona P, Aljada A. Advances in diabetes for the millennium: diabetes and the endothelium. Med Gen Med. 2004;6(3):6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1474826/.

[9] Pillai S, Davies G, Lawrence M, Whitley J, Stephens J, Williams PR, Morris K, Evans PA. The effect of diabetic ketoacidosis (DKA) and its treatment on clot microstructure: Are they thrombogenic? Clin Hemorheol Microcirc. 2021;77(2):183-94. doi: 10.3233/CH-200957.

[10] American Diabetes Association. Physical activity/exercise and diabetes. Diabetes care. 2004;27(1):58-62. doi.org/10.2337/diacare.27.2007.S58.

[11] Joseph A, Parvathy S, Varma KK. Hyperinsulinemia Induced Altered Insulin Signaling Pathway in Muscle of High Fat- and Carbohydrate-Fed Rats: Effect of Exercise. J Diabetes Res. 2021:5123241. doi: 10.1155/2021/5123241.

[12] Micalos PS, Marino FE, Tarpenning K, Kay D, Gard M. Ammonia and lactate responses to isokinetic arm and leg exercise. Isokinet Exerc Sci. 2001;9(2-3):143-9. doi:10.3233/IES-2001-0069.

[13] Medawar PB. The definition and measurement of senescence. Ciba Found Symp. 1955;1(1):4-15. doi.org/10.1002/9780470718926.ch2.
[14] Ahmadizad S, El-Sayed MS, MacLaren DP. Responses of platelet activation and function to a single bout of resistance exercise and recovery. Clin Hemorheol Microcirc. 2006;35(1-2):159-68. https://europepmc.org/article/med/16899922.
[15] Ahmadizad S, El-Sayed MS. The effects of graded resistance exercise on platelet aggregation and activation. Med Sci Sports Exerc. 2003;35(6):1026-32. doi: 10.1249/01.mss.0000069406.54766.c6.
[16] Kraemer WJ, Adams K, Cafarelli E, Dudley GA, Dooly C, Feigenbaum MS. Progression models in resistance training for healthy adults. American college of sports medicine. Med Sci Sports Exerc. 2002;34(2):364-80. doi: 10.1097/00005768-200202000-00027.
[17] Hughes L, Paton B, Rosenblatt B, Gissane C, Patterson SD. Blood flow restriction training in clinical musculoskeletal rehabilitation: a systematic review and meta-analysis. Br J Sports Med. 2017;51(13):1003-11. https://dx.doi.org/10.1136/bjsports-2016-097071.
[18] Loenneke JP, Wilson GJ, Wilson JM. A mechanistic approach to blood flow occlusion. Int J Sports Med. 2010;31(01):1-4. doi: 10.1055/s-0029-1239499.
[19] Bond CW, Hackney KJ, Brown SL, Noonan BC. Blood flow restriction resistance exercise as a rehabilitation modality following orthopaedic surgery: a review of venous thromboembolism risk. J Orthop Sports Phys Ther. 2019;49(1):17-27. doi: 10.2519/jospt.2019.8375.
[20] Madarame H, Kurano M, Takano H, Iida H, Sato Y, Ohshima H, et al. Effects of low-intensity resistance exercise with blood flow restriction on coagulation system in healthy subjects. Clin Physiol Funct Imaging. 2010;30(3):210-3. doi.org/10.1111/j.1475-097X.2010.00927.x.
[21] Cauza E, Hanusch-Enserer U, Strasser B, Ludvik B, Metz-Schimmerl S, Pacini G, et al. The relative benefits of endurance and strength training on the metabolic factors and muscle function of people with type 2 diabetes mellitus. Arch Phys Med Rehabil. 2005;86(8):1527-33. doi.org/10.1016/j.apmr.2005.01.007
[22] Costill DL, Fink WJ. Plasma volume changes following exercise and thermal dehydration. J Appl Physiol. 1974;37(4):521-5. doi.org/10.1152/jappl.1974.37.4.521.
[23] Rocker L, Gunay S, Gunga HC, Hopfenmüller W, Ruf A, Patscheke H, et al. Activation of blood platelets in response to maximal isometric exercise of the dominant arm. Int J Sports Med. 2000;21(03):191-4. doi: 10.1055/s-2000-303.
[24] El-Sayed MS, Ali ZE, Ahmadizad S. Exercise and training effects on blood haemostasis in health and disease. Sports Med. 2004;34(3):181-200. doi: 10.2165/00007256-200434030-00004.
[25] McDonald TP, Cottrell M, Clift R. Effects of short-term hypoxia on platelet counts of mice. Blood. 1978;51(1):165-75. doi.org/10.1182/blood.V51.1.165.165.
[26] Ozaki H, Miyachi M, Nakajima T, Abe T. Effects of 10 weeks walk training with leg blood flow reduction on carotid arterial compliance and muscle size in the elderly adults. Angiology. 2011;62(1):81-6. doi.org/10.1177/0003319710375942.
[27] Pope ZK, Willardson JM, Schoenfeld BJ. Exercise and blood flow restriction. J Strength Cond Res. 2013;27(10):2914-26. doi: 10.1519/JSC.0b013e3182874721.
[28] Petridou A, Chatzinikolaou A, Fatouros I, Mastorakos G, Mitrakou A, Chandrinou H, et al. Resistance exercise does not affect the serum concentrations of cell adhesion molecules. Br J Sports Med. 2007;41(2):76-9. http://dx.doi.org/10.1136/bjsm.2006.031047.
[29] Smith LL, Anwar A, Fragen M, Rananto C, Johnson R, Holbert D. Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. Eur J Appl Physiol. 2000;82(1):61-7. doi: 10.1007/s004210050652.
[30] Deaglio S, Robson SC. Ectonucleotidases as regulators of purinergic signaling in thrombosis, inflammation, and immunity. Adv Pharmacol. 2011;61:301-32. doi: 10.1016/B978-0-12-385526-8.00010-2.
[31] Grenz A, Homann D, Eltzschig HK. Extracellular adenosine: a safety signal that dampens hypoxia-induced inflammation during ischemia. Antioxid Redox Signal. 2011;15(8):2221-34. doi: 10.1089/ars.2010.3665.
[32] Haskó G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat Rev Drug Discov. 2008;7(9):759-70. doi: 10.1038/nrd2638.
[33] Burnstock G. Purinergic signaling in the cardiovascular system. Circ Res. 2017;120(1):207-28. doi: 10.1161/CIRCRESAHA.116.309726.
[34] Gao Y. Conventional and unconventional mechanisms for soluble guanylyl cyclase signaling. J Cardiovasc Pharmacol. 2016;67(5):367-72. doi: 10.1097/FJC.0000000000000330.
[35] Tousoulis D, Kampoli AM, Tentolouris Nikolaos Papageorgiou C, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol. 2012;10(1):4-18. doi: 10.2174/157016112798829760.
[36] Downs ME, Hackney KJ, Martin D, Caine TL, Cunningham D, O'Connor DP, et al. Acute vascular and cardiovascular responses to blood flow-restricted exercise. Med Sci Sports Exerc. 2014;46(8):1489-97. doi: 10.1249/MSS.0000000000000253.

[37] Ji JW, Mac Gabhann F, Popel AS. Skeletal muscle vegf gradients in peripheral arterial disease: simulations of rest and exercise. Am J Physiol-Heart C. 2007;293(6):3740-9. doi: 10.1152/ajpheart.00009.2007.
[38] Berckmans RJ, Nieuwland R, Böing AN, Romijn FP, Hack CE, Sturk A. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. J Thromb Haemost. 2001;85(04):639-49. doi: 10.1055/s-0037-1615646.
[39] Bittencourt CR, Izar MC, França CN, Schwerz VL, Póvoa RM, Fonseca FA. Effects of chronic exercise on endothelial progenitor cells and microparticles in professional runners. Arq Bras Cardiol. 2017;108(3):212-6. doi: 10.5935/abc.20170022.
[40] Guiraud T, Gayda M, Juneau M, Bosquet L, Meyer P, Théberge-Julien G, et al. A single bout of high-intensity interval exercise does not increase endothelial or platelet microparticles in stable, physically fit men with coronary heart disease. Can J Cardiol. 2013;29(10):1285-91. doi: 10.1016/j.cjca.2013.03.024.
[41] Hilberg T, Menzel K, Gläser D, Zimmermann S, Gabriel HH. Exercise intensity: platelet function and platelet–leukocyte conjugate formation in untrained subjects. Thromb Res. 2008;122(1):77-84. doi: 10.1016/j.thromres.2007.08.018.
[42] Wang JS. Intense exercise increases shear-induced platelet aggregation in men through enhancement of von willbrand factor binding, glycoprotein iib/iiia activation, and p-selectin expression on platelets. Eur J Appl Physiol. 2004;91(5):741-7. doi: 10.1007/s00421-004-1050-4.
[43] Lu Q, Malinauskas RA. Comparison of two platelet activation markers using flow cytometry after *in vitro* shear stress exposure of whole human blood. Artif Organs. 2011;35(2):137-44. doi: 10.1111/j.1525-1594.2010.01051.x.
[44] Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, Green DJ. Shear stress mediates endothelial adaptations to exercise training in humans. Hypertension. 2010;55(2):312-8. doi: 10.1161/HYPERTENSIONAHA.109.146282.
[45] Ando J, Yamamoto K. Effects of shear stress and stretch on endothelial function. Antioxid Redox Signal. 2011;15(5):1389-403. doi: 10.1089/ars.2010.3361.
[46] Wurzinger LJ, Opitz R, Wolf M, Schmid-Schönbein H. Shear induced platelet activation-a critical reappraisal. Biorheology. 1985;22(5):399-413. doi: 10.3233/bir-1985-22504.