

The Effect of Regular Physical Activity on Platelet Activity in Men of Mature Age

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Received: 29 January 2023 / Received in revised form: 20 May 2023, Accepted: 27 May 2023, Published online: 15 June 2023

Abstract

At present, much attention is paid to various aspects concerning the impact of systematic muscular load on platelet parameters. In our study, we examined 107 healthy mature males. They were divided into 4 groups. In this study, we determined the mean platelet volume, their aggregation activity when introduced into plasma with 2.5, 5, and 10 μM ADP, and the amount of GP IIb-IIIa and GP Ib on the platelet surfaces. With an increase in daily muscle activity, the examined patients showed a weakening in the process of platelet aggregation in relation to all the applied concentrations of ADP, the content of adenosine phosphates in them, and the secretion of adenosine phosphates from them, the platelet level of actin and myosin, a decrease in platelets size and a reduction in the GP IIb-IIIa and GP Ib expressiveness. There was revealed the correlation between the volume of platelets, on the one hand, and platelet aggregation, the adenosine phosphates content in them and secretion from them, the level of actin and myosin self-assembly, and the density of GP IIb-IIIa and GP Ib receptors on platelet membranes, on the other hand, which, to a greater extent, manifested itself in the most physically active people. The changes in platelet parameters that occur in males of the second mature age if the distance traveled per day increases indicate the possibility of their optimization through a rational and controlled increase in physical activity within a day.

Keywords: Platelets, Muscle activity, Regular exercise, Platelet

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Introduction

Despite their small size, platelets are biologically important blood corpuscles participating in many processes of body balance control (Zavalishina, 2018a; Vorobyeva *et al.*, 2020). Platelets show their biological activity during their adhesion, aggregation, and secretion (Zavalishina, 2018b). Platelet functions may be congenitally impaired. An example of this is sticky platelet syndrome, in which there is platelet hyperreactivity in vitro to adenosine diphosphate and adrenaline. These people often develop arterial and venous thrombosis (Sokol *et al.*, 2018). When there occur negative changes in the external environment and the body, platelets very often increase their activity with the signs of thrombophilia (Sacramento *et al.*, 2020; Karpov *et al.*, 2021a). In this regard, modern researchers continue to study the mechanisms for platelet function implementation due to various effects on the body (Gutiérrez-Herrero *et al.*, 2020; Zavalishina *et al.*, 2022). Much attention has always been paid to the severity of platelet aggregation and its size, as those parameters comprehensively reflect their functional abilities (Klovaite *et al.*, 2011; Jongen *et al.*, 2020). Fibrinogen plays an important role in the process of platelet aggregation. It serves as a framework for the development of platelet aggregation resulting from its interaction with platelet integrin $\alpha\text{IIb}\beta\text{3}$ (glycoprotein IIb/IIIa) (Simurda *et al.*, 2021).

The number of GP IIb-IIIa (Yakushkin *et al.*, 2011; Kunicki *et al.*, 2012) and GP VI (Roest *et al.*, 2000; Joutsu-Korhonen *et al.*, 2003) receptors located on their membranes, which largely determine platelet activity in case of some changes in a person's general condition, is considered to be very important for the aggregation process implementation by platelets.

There was revealed a relationship between the number of glycoprotein IIb-IIIa (Sirotkina *et al.*, 2007), glycoprotein VI (Furihata *et al.*, 2001; Catani *et al.*, 2020) molecules located on platelets and the severity of platelet functions. Therefore, the amount of these proteins on the surface of platelets is recognized as an important marker of their overall functional activity (Döhrmann *et al.*, 2020; Marín Oyarzún *et al.*, 2020). The presence of a relationship between the density of glycoprotein molecules that provide adhesion on the surfaces of platelet membranes and the surface area of platelets containing these proteins was noted. It is clear that the larger the platelets, the more actively they



aggregate, the more they contain granules with biologically active substances intended for secretion, and the more they contain the substances necessary for the implementation of homeostasis (Wong *et al.*, 1989). Therefore, an increase in platelet surface area is considered dangerous when we mean the conditions for the development of platelet hyperfunction (Zavalishina, 2018c; Yoshida *et al.*, 2020).

In the human body in adulthood, primarily in males, the degree of pathological burden gradually increases. This is largely due to the appearance and progression of the subclinical atherosclerosis phenomena, gradually increasing episodes in blood pressure rise or persistent arterial hypertension development with increased hemostatic properties of platelets, as well (Goïta *et al.*, 2020; Liu *et al.*, 2020). These moments are very unfavorable prognostic allies due to the occurrence of thrombophilia, which often results in death or disability (Karpov *et al.*, 2020; Ruscica *et al.*, 2020).

Hence, modern researchers are rather interested in identifying the approaches to physiologically reduce the level of platelet function activity, especially in males of the second mature age (Kotova *et al.*, 2017; Rolnik *et al.*, 2020). According to various studies, a very effective element of preventive measures to reduce the thrombophilia phenomena in people who do not have signs of obvious pathology is considered to be non-drug effects (Zavalishina *et al.*, 2021a). One of them is regular physical activity adequate for the human condition (Mal *et al.*, 2018). It has been noted that they cause very positive changes in all vital organs, providing an optimal level of viability for the whole organism (Fayzullina *et al.*, 2020). Systematic physical activity also has a very positive effect on blood parameters, humoral regulation, and trophic processes in tissues (Vorobyeva *et al.*, 2018). However, the peculiarities concerning the changes in the functional parameters of platelets due to an increase in daily physical activity have not been fully assessed. This requires additional observations of different categories of people under conditions of regular physical activity (Bespalov *et al.*, 2018).

It was established in some earlier studies that in the presence of body dysfunctions it is possible to reduce platelets' functional manifestations with an increase in physical activity at a young age (Savchenko, 2013). However, the effect of high muscular activity on platelet size as a result of walking in clinically healthy males of the second maturity age needs further study. It is still unexplained how different distances covered daily on foot affect the activity of the main hemostatic manifestations of platelets in males of the second mature age. The reaction of platelet aggregation, in males belonging to this age group in particular, to daily walks at a distance over their daily demands needs to be clarified.

The aim of the research: is to assess how an increase in daily muscular activity affects the functional characteristics of platelets in mature males.

Materials and Methods

The study was performed on 107 clinically healthy males in their second adulthood (mean age 41.2 ± 1.9 years), who did not suffer from any chronic diseases and did not have bad habits. The study

was conducted from 01/01/2018 to 12/31/2019. Taking into account the level of daily muscular activity of the surveyed, 4 groups were collected. The control group of the observed is represented by 25 individuals who experience only household stress during their lives and who avoided physical culture and sports. Study group 1 included 27 people who, in addition to household activities, for at least 1 year were engaged in race walk in a free mode and walked at least 3 kilometers a day. Study group 2 consisted of 26 people who, in addition to everyday activities, were engaged in daily race walk for at least 1 year. They walked at least 5 kilometers a day in free mode. Study group 3 included 29 people who, in addition to everyday activities, were engaged in race walking for at least 1 year, walking at least 8 kilometers a day.

In the subject males, blood was taken from the cubital vein in a 3.8% solution of sodium citrate with the achievement of the final ratio of blood and the applied anticoagulant - 9:1. Platelet-rich plasma was obtained after centrifugation of blood for 10 minutes at 180 g at room temperature. Platelet-poor plasma was obtained by centrifuging platelet-rich plasma for 15 minutes at room temperature at 1500 g. The average volume of platelets in the blood, the amount of adenosine diphosphate and adenosine triphosphate in them, the degree of adenosine phosphates secretion from platelets, the actin and myosin level in platelets, the degree of platelet aggregation, and the number of GPIb and GPIIb-IIIa receptor molecules located on the platelet surfaces were determined in all those taken into the study. All these parameters were assessed within the first hour after taking blood from the vein.

The mean platelet volume and the number of platelet bodies present in the platelet-rich plasma were determined using the AbacusJuniorB hematology analyzer (DiatronLtd, Austria).

A quantitative assessment of the ATP and ADP content in platelets was carried out, and the severity of their secretion was determined in response to the addition of collagen in a standard concentration to the medium with platelets (Ermolaeva *et al.*, 1992).

There were assessed actin and myosin levels in intact platelets and the presence of platelet activation and aggregation, develop in response to the ADP and thrombin introduction in standard concentrations into the medium with platelets (Ermolaeva *et al.*, 1992).

In the course of the study, we determined the level of platelet aggregation stimulated during the work by the ADP inducer. This was carried out using a standard turbid metric method with an assessment of the severity of the changes in light transmission in platelet-rich plasma (T%). For this purpose, a platelet aggregation analyzer was used, which has the BIOLA trademark (manufactured by BIOLA, Russia). The device ensured that the plasma remained at 37°C in the sample and was stirred at a high speed (800 rpm). The ADP inducer was used during the study at three final concentrations of 10 µM, 5 µM, and 2.5 µM. It was added to platelet-rich plasma exactly in 30 seconds following the start of the light transmission assessment, then assessing it for another 4.5 minutes after the entry of ADP into the plasma. On platelet aggregation curves, the severity of aggregation was recorded (T% maximum value).

The assessment of the GP Ib and GP IIb-IIIa content on platelet surfaces was performed following traditional proven methods (Sirotkina *et al.*, 2007; Yakushkin *et al.*, 2011) by standard labeled monoclonal antibodies that interact with these proteins. Platelet-rich plasma was standardized for platelet content using autologous plasma to a value of 250000/microliter. In case of a lower platelet content in the plasma, autologous platelet-rich plasma was added to it. Registration of the GP IIb-IIIa count on platelets was carried out using CRC64 antibodies specific to it (Mazurov *et al.*, 1991). To determine the GP Ib level, VM16d antibody was used. These labeled antibodies were separately added to platelet-rich plasma samples, achieving their final amount of 10 µg/ml in the absence or presence of a twenty-fold excess of unlabeled antibodies (general and non-specific binding was detected, respectively). Platelets were incubated in plasma together with antibodies for 30 minutes at 37°C. At the end of the incubation, the platelet-bound label and the label that did not bind to platelets were separated from each other by centrifuging a platelet-rich plasma sample with a 20% sucrose solution. Specific binding was determined by subtracting non-specific binding from the total binding level. The obtained value of glycoproteins was considered to be the number of antibody molecules capable of binding to one platelet.

Plasma fibrinogen levels were assessed using the traditional method.

Mathematical processing of the indicators obtained in the course of the research was carried out using the computer program “Statistica 10.0 for Windows”. In the course of the study, non-parametric data processing methods were used: the Mann-Whitney U-test, Fisher's F-test of reliability (statistical significance was determined at a value of $p < 0.05$), and the Spearman rank correlation method.

Results and Discussion

Features of the Mean Platelet Volume

The value of the average platelet volume in physically untrained men was higher than in men who increased their muscle activity through sports walking. The average value and the range of fluctuations of their volume values were within the limits of the optimum. In men who made up the control group, the value of the average platelet volume ranged from 7.2 to 10.9 fl. In those experiencing regular physical activity, this indicator ranged from 5.7 to 10.2 fl.

In the conducted study we managed to find significant differences in the values of the mean platelet volume between the control group and those subjects who made up the study groups. Moreover, as the distance traveled per day increased, the differences in this platelet index in males increased as well. Thus, in the most physically active males from study group 3, the differences from the control were most significant.

The use of one-way ANOVA showed that in the study groups, there is a relationship between the value of the mean platelet volume and the sports distance traveled during the day. Because the obtained F value exceeds the F critical (3.120), the null hypothesis is completely excluded.

The Ability of Platelets to Aggregate

In the observation performed, platelet aggregation in all the subjects participating in the research was stimulated with the ADP inducer at various concentrations. In all the examined males, platelet aggregation indicators increased with an increase in the concentration of this inductor (**Table 1**).

Table 1. Platelet Aggregation in Those Subjects Participating in the Examination

Study groups	T, % being influenced by ADP at a dose of 2.5 µM	T, % being influenced by ADP at a dose of 5 µM	T, % being influenced by ADP at a dose of 10 µM
Control group	38.2±1.5	53.1±1.7	63.9±2.2
Study group 1	35.6±1.3	47.8±1.6	56.7±1.7*
Study group 2	31.8±0.6**	45.0±1.2*	54.6±1.3*
Study group 3	28.5±0.8** F= 4.846 ($p \leq 0.0051$)	41.1±0.7** F= 5.138 ($p \leq 0.0073$)	50.2±0.8** F= 5.623 ($p \leq 0.0079$)

Note. Statistical significance of differences in platelet aggregation parameters in groups that started physical training compared to the control group:

* - $p < 0.05$, ** - $p < 0.01$.

The most significant platelet aggregation, which develops under the influence of all the inductor counts applied, was revealed in the control group, which included males who consciously avoided significant physical exertion. In the first study group, the severity of platelet aggregation appeared to be less. However, the differences in this group concerning this indicator from the control parameters turned out to be significant only in the case of the maximum concentration of the inductor used (12.6%). Platelet aggregation activity stimulated by ADP was less in those males who additionally walked 5 km daily. Its values were significantly lower than the control in response to all the inductor concentrations

applied. The activity of platelet aggregation in males additionally passing 8 km per day is, to a greater extent, at a disadvantage in comparison with the control values. In the third study group, in the case of the inductor minimum concentration, the level of platelet aggregation was 34.0% lower than that of the control parameters, in the case of the concentration at 5 µM it was 29.2% lower than that of the control, and in case of the concentration at 10 µM it was 27.3% lower than the control parameters. The significance of differences from the parameters in the control group concerning the value of platelet aggregation that occurred in response to all the

tested inductor concentrations was the greatest in the third study group.

Processing of platelet aggregation values utilizing one-way analysis of variance showed that in the study groups, there was a relationship between platelet aggregation activity in response to all the concentrations of the inductor applied and the distance covered by the athletes within a day. Because the obtained F values exceed F critical for each inductor concentration (F critical for ADP 2.5 μM is 3.254; F critical for ADP 5 μM is 3.567; F critical for ADP 10 μM is 3.884), we can assume that there are differences in the degree of aggregation between the study groups and that the null hypothesis can be completely rejected.

The content of the protein fibrinogen in the plasma is considered very important for the implementation of platelet aggregation. In the male who made up the control group, its concentration was

4.4 \pm 0.35 g/l. When the distance covered daily on foot increased, the content of fibrinogen in the blood of the males being examined decreased. In the first study group, its concentration was 3.7 \pm 0.37 g/l, in the second study group - 3.4 \pm 0.29 g/l, and in the third study group - 3.1 \pm 0.22 g/l.

In the males of the control sample and those from the study groups, we revealed correlations that were traced between the parameters of the platelet aggregation index and those of the mean platelet volume (Table 2). Significant correlations were revealed in the representatives of the control group in relation to all the tested ADP inductor concentrations. In the males included in the study groups, regardless of the distance they covered a day, there was a correlation between the mean platelet volume and the activity of platelet aggregation stimulated by adenosine diphosphate at 5 μM and 10 μM . In case its concentration was 2.5 μM , the correlation was unreliable.

Table 2. Correlation between the State of Platelet Aggregation and the Mean Platelet Volume

Study groups participating in the research	Correlation coefficients when comparing platelet aggregation and mean platelet volume		
	ADP 10 μM	ADP 5 μM	ADP 2,5 μM
Studygroup 1, n = 27	0.59*	0.62**	0.32
Study group 2, n = 26	0.60**	0.59**	0.35
Study group 3, n = 29	0.61**	0.63**	0.34
Controlgroup, n = 25	0.57*	0.59**	0.38*

Note. The confidence level in the correlation coefficient: * - $p < 0.05$; ** - $p < 0.01$.

Glycoproteins of Surface Platelet Membranes

Information about the content of GPIb and GPIIb-IIIa on the platelet membranes of the males participating in the study and fluctuations in their number are shown in Table 3. In subjects who increased their level of physical activity and in physically poorly trained, there were noted very wide variations in the levels of GPIIb-IIIa and GPIb on platelet membranes.

In the study groups, a decrease in the expression of these two glycoproteins was noted, if the distance covered on foot within a day increased. This contributed to significant differences in the amount of both recorded glycoproteins between the second and third study groups, who covered on foot the longest distances within a day, and the control group (Table 3).

Table 3. The Number of Registered Glycoproteins on the Outer Surface of Platelets in Subject Males

Platelet glycoproteins	First study group, n = 27	Second study group, n = 26	Third study group, n = 29	Control group, n = 25
GPIIb-IIIa, 10 ³ for 1 platelet	45.7 \pm 2.6 (30.0-62.0)	42.0 \pm 2.4* (28.0-60.0)	39.4 \pm 2.8** (27.0-57.0) F= 4.672 ($p \leq 0.0071$)	48.6 \pm 2.0 (34.0-65.0)
GPIb, 10 ³ for 1 platelet	24.9 \pm 2.2 (15.0-34.0)	23.1 \pm 1.7* (14.0-32.0)	21.3 \pm 1.5** (13.0-30.0) F= 4.372 ($p \leq 0.0083$)	26.9 \pm 1.6 (18.0-37.0)

Note. Significance of differences in values between the study and control groups: * - $p < 0.05$, ** - $p < 0.01$.

Having processed the quantitative content of the considered receptors on platelet membranes by one-way ANOVA, we revealed that in the study groups, there was a relation between the f GPIIb-IIIa and GPIb count on platelets and the distance covered by sportsmen on foot during a day. Because the obtained F values exceed F critical for each of the receptors being assessed (F critical for GPIIb-IIIa is 3.146; F critical for GPIb is 3.682), we can claim

that there are differences in the number of these receptors on the platelets surface between the study groups and the null hypothesis can be completely rejected.

The GPIb and GPIIb-IIIa count and the mean platelet volume in the subject males were related to each other. In the males who took up physical training, regardless of the distance they walked during

the day, and in those from the control group, there occurred a statistically significant correlation between the GPIIb-IIIa and GPIIbcount and the mean platelet volume. At the same time, in all

the groups, a statistically significant correlation was marked between the levels of both glycoproteins of platelet membranes - r from 0.51 to 0.58 (Table 4).

Table 4. The Values of the Correlation Coefficients between the Level of Platelet Glycoproteins under Consideration and the MeanPlatelet Volume

Correlationpairs	First studygroup, n = 27	Second studygroup, n = 26	Third studygroup, n = 29	Controlgroup, n = 25
	Correlation coefficient values			
GP IIb-IIIa/ meanplateletvolume	0.61**	0.63**	0.66**	0.59**
Meanplateletvolume/ GPIIb	0.58*	0.60**	0.55*	0.53*
GPIIb / GP IIb-IIIa	0.55*	0.57*	0.58*	0.51*

Note. Statistical significance of correlation coefficients: * - p <0.05, ** - p <0.01.

Platelet Actin and Myosin

The level of actin in intact platelets in physically active males was lower than that in the control group. Moreover, it decreased when physical activity increased, accounting for 20.2±0.07% of the total protein count in the platelet in the third study group. As for the degree of actin additional formation in presence of platelet activation by a strong or weak inductor and during further platelet aggregation in physically training subjects, this indicator turned out to the lower, the longer the daily distance covered on foot.

Processing of the actin quantitative content in platelets through one-way ANOVA showed that in the study groups, there was a relation between its amount in platelets of varying degrees of activation and the distance covered by athletes during the day.

Because the obtained F values exceeded the F critical for each of the indicators assessed, we can claim that there are differences in actin count in intact and activated platelets between the study groups. The above-mentioned provides us a basis to completely reject the null hypothesis.

Actin count in intact and active platelets and the mean platelet volume were related in the males participating in the study. In males who regularly experienced physical exertion, regardless of the distance they traveled a day, and in those who refused regular physical exertion, the values of the correlation coefficients (r) were statistically significant during the process of platelet aggregation when using a weak and strong inductor. At the same time, in all the groups, the noted statistically significant correlation turned out to be direct and of average force - r from 0.52 to 0.63 (Table 5).

Table 5. The Value of the Correlation Coefficients between the Actin Level in Platelets and the Mean Platelets Volume in the Examined Males

Actincount in platelets	First studygroup, n = 27	Second study group, n = 26	Third study group, n = 29	Controlgroup, n = 25
	Correlation coefficient values			
intact, % tothe total proteincount in theplatelet	0.32	0.34	0.37	0.41
in presence of ADP activation,% tothe total proteincount in theplatelet	0.41	0.39	0.36	0.44
in presence of ADP aggregation,% tothe total proteincount in theplatelet	0.56*	0.55*	0.52*	0.58*
in presence of thrombinactivation,% tothe total proteincount in theplatelet	0.45	0.42	0.43	0.48
in presence ofthrombinaggregation,% tothe total proteincount in theplatelet	0.59*	0.55*	0.52*	0.63**

Note. Statistical significance of the correlation coefficients obtained in the study: * - p <0.05, ** - p <0.01.

Similar features of the functional activity in the platelets of the subject males were found for the myosin mechanism. It was remarked that in intact platelets of physically active males, the myosin level fell short of the control level (18.2±0.17% of the total protein content in the platelet), having the lowest level in the third study group (13.2±0.08% of the total protein content) protein in the platelet. This pattern remained even in presence of platelet activation by strong or weak inductors and with their further

aggregation in all the subjects participating in the study. When the level of muscular activity increased there observed a decrease in the degree of myosin formation in blood platelets in the context of platelets participation in hemostasis.

Processing of the quantitative myosin content in platelets using one-way ANOVA revealed that in the study groups, there was a relationship between its amount in platelets of different activation degrees and the distance covered on foot by sportsmen during the

day. Because the obtained F values exceeded the F critical for each of the indicators assessed, it can be considered that there are differences in the myosin count in intact and activated platelets between the study groups. The above-mentioned fact provides a basis to completely reject the null hypothesis.

The myosin count in platelets and the mean platelet volume were related to each other in the males participating in the study. In those

who deliberately increased their level of physical activity, regardless of the distance they covered on foot a day, and in the control group, the values of the correlation coefficients between the myosin count and the mean platelet volume were statistically significant in presence of platelet aggregation caused by a weak and a strong inducer. At the same time, in all the groups, the observed statistically significant correlation was direct and had an average force - r from 0.52 to 0.64 (**Table 6**).

Table 6. The Value of the Correlation Coefficients between the Myosin Level in Platelets and the Mean Platelets Volume in the subject males

Myosin count in platelets	First studygroup, n = 27	Secondstudygroup, n = 26	Third studygroup, n = 29	Controlgroup, n = 25
	Correlation coefficient values			
intact,% to the total protein count in the platelet	0.30	0.32	0.28	0.39
in presence of ADP activation,% to the total protein count in the platelet	0.42	0.36	0.33	0.47
in presence of ADP aggregation,% to the total protein count in the platelet	0.58*	0.55*	0.52*	0.63**
in presence of thrombin activation,% to the total protein count in the platelet	0.47	0.44	0.40	0.50
in presence of thrombin aggregation,% to the total protein count in the platelet	0.60**	0.55*	0.53*	0.64**

Note. Statistical significance of the correlation coefficients obtained in the study: * - $p < 0.05$, ** - $p < 0.01$.

ATP and ADP of Platelets

In the control group, the ATP and ADP count in the males' platelets turned out to be the highest ($5.96 \pm 0.016 \mu\text{mol}/10^9$ platelets and $3.92 \pm 0.015 \mu\text{mol}/10^9$ platelets, respectively). At the same time, the level of the ATP and ADP secretion from platelets in males from the control group was also maximum ($40.7 \pm 0.19\%$ and $42.8 \pm 0.8\%$, respectively).

In those participants who exercise, the amount of ATP and ADP in platelets was lower, the higher their daily physical activity was. These indicators were the lowest in the third study group, accounting for $5.06 \pm 0.014 \mu\text{mol}/10^9$ platelets and $3.10 \pm 0.009 \mu\text{mol}/10^9$ platelets, respectively. The males of this group also had the least active ATP and ADP secretion from platelets, accounting for $30.3 \pm 0.14\%$ and $32.1 \pm 0.10\%$, respectively.

Processing of the quantitative ATP and ADP content in platelets and the degree of their secretion from platelets utilizing one-way ANOVA revealed the relationship in the study groups between the level of these platelets and the distance traveled on foot by the

sportsmen within a day. Since the obtained F values exceeded the critical F for each of the indicators assessed, we can assume that there are differences in the ATP and ADP count in platelets and the degree of adenosine phosphates secretion from them between the study groups. The above-mentioned fact provides us a basis to completely reject the null hypothesis.

The thrombocytic count of ATP and ADP and the mean platelet volume in the subject males were related to each other. In physically active males, despite the distance, they cover a day, and in those who experience only household physical exertions, between the ATP and ADP content in platelets and the mean platelet volume, the values of the correlation coefficients were statistically significant and ranged from 0.54 to 0.63. They increased from the first group to the third one, corresponding to the average correlation strength. At the same time, in all the groups of the examined subjects, there was noted a statistically significant direct correlation of the average force between the level of adenosine phosphates secretion and the volume of the mean platelet in their blood. The highest correlation coefficients were found in the third study group (**Table 7**).

Table 7. The Value of the Correlation Coefficients between the Values of Individual Functional Indicators of Platelets and Their Mean Volume in the Examined Males

Platelet counts	First studygroup, n = 27	Secondstudygroup, n = 26	Third studygroup, n = 29	Controlgroup, n = 25
	Correlation coefficient values			
ATP content in platelets before the secretion, $\text{mol}/10^{11}$ platelets	0.60**	0.62**	0.65**	0.56**
ADP content in platelets before secretion, $\text{mol}/10^{11}$ platelets	0.57*	0.61**	0.63*	0.54*

Level of ATP secretion from platelets, %	0.58**	0.60**	0.65**	0.55*
Level of ADP secretion from platelets, %	0.60**	0.62**	0.64**	0.57**

Note. Statistical significance of the correlation coefficients obtained in the study: * - $p < 0.05$, ** - $p < 0.01$.

When performing the research among the males of the second mature age, who walked daily in a free mode, we managed to take an assessment of the following parameter: the mean platelet volume, the state of the ability of the platelets to aggregate, the ATP and ADP count and the degree of their secretion, the actin and myosin levels, and the platelets GP IIB-IIIa and GP Ib count located on membranes. The research was carried out in groups staying within the limits of the previously agreed level of physical activity. As for a group of comparison, we considered a group of healthy physically inactive volunteers of the same age.

Having assessed the mean platelet volume and the GP Ib and GP IIB-IIIa count, there was determined a certain variability of these parameters in groups with increased levels of physical activity and a group with low physical activity. In our research, we managed to reveal the differences in the GP Ib and GP IIB-IIIa levels and the value of the mean platelet volume between the examined untrained males and those males who increased their physical activity. Clear selection in the study groups and strict performance of physical activity made it possible to find out. The data obtained indicate there is a relation between these parameters in the males of the second mature age and the distance they walk a day. It was noted early in this paper that clinically healthy people leading a healthy lifestyle are characterized by a consistently low level of GP IIB-IIIa expression (Haspekova *et al.*, 2008). However, this study did not find any association between the amount of this receptor and the daily level of physical activity.

In the males from the control group the level of the average platelet size ranged between 7.2 and 10.9 fl, while in those who increased their physical activity, it ranged from 5.7 to 10.2 fl. Significant differences were revealed between the average size of platelets in the control group and the groups involved in the race walk. Resulting of the increase in the distance traveled a day, there was observed a reduction in the differences between the average level of this parameter. Therefore, in the most physically active males who were in the third study group, these differences with the control turned out to be the most significant. The obtained information can be explained by physiologically beneficial changes in the bone marrow work in the context of a persistent increase in physical activity (Zavalishina, 2020; Dorontsev *et al.*, 2022).

The greatest degree of platelet aggregation when adding all the inducer concentrations to the plasma was pointed out in physically untrained males who made up the control group. In the first study group, the level of platelet aggregation was lower. At the same time, in this group, the level of aggregation significantly differed from its level in the control one only when the highest inducer concentration (11.0%) was used. Stimulated platelet aggregation appeared to be lower in the males who walked 5 kilometers a day. Its values were significantly less than the control level in relation to all the applied concentrations of adenosine diphosphate. The values of platelet aggregation in males who walked the maximum distance during the day, to a greater extent, fell short of the control

values. Thus, in the third study group, due to ADP use at 2.5 μM , the value of platelet aggregation was lower by 34.0% than its indicator in the control, due to a concentration at 5 μM , it was lower than the control values by 29.2%, and due to the inducer application at 10 μM , it appeared to be less than the control level by 27.3%. Thus, in the third study group, the degree of platelet aggregation induced by all the concentrations of the inducer used in the study differed, to the greatest extent, from the aggregation level in the control.

In the research, it became possible to identify in physically active males direct statistically significant correlations between the mean platelet volume and the severity of their aggregation. In all the examined groups that experienced regular physical activity, we managed to find significant, moderate correlations for ADP doses of 5 and 10 μM . When administered at a dose of 2.5 μM in the study groups, there was a tendency to stay within the level of statistical significance. The correlation was statistically significant with this ADP concentration only in the control group. It was mentioned above that a reasonable increase in the volume of physical activity results in the development of a gradual biologically very beneficial decrease in the P2Y12 receptor expression in platelets, which can interact with ADP. This can explain the revealed weakening in the process of platelet ADP aggregation as daily physical activity increases.

There are reasons to think that systematic aerobic exercises can simultaneously reduce the mean platelet volume and inhibit the severity of their ADP-stimulated aggregation. The degree of reduction in platelet aggregation is very strongly associated with the level of physical activity, in presence of which the number of glycoproteins IIB-IIIa decreases on the platelet membranes. An earlier study (Khaspekova *et al.*, 2011) showed a direct correlation between the severity of its expression and the activity of ADP platelet aggregation. However, the search for its relationship with the level of daily physical activity experienced by the subjects was not performed in this research.

The present study made it possible to find a significant correlation of medium force between the GP IIB-IIIa density on the platelet's surface and the mean platelet volume. This was observed in the males leading a physically active lifestyle and taking daily walks at different distances. The data obtained in the study concerning the relationship between the count of GP IIB-IIIa platelets and the mean platelet volume are confirmed by previously obtained data (Kunicki *et al.*, 2012).

In the males who are weakly physically trained and consciously avoiding physical activity, we managed to find a clear relation between the mean platelet volume and the number necessary for the implementation of their adhesion to GP Ib molecules. At the same time, with an increase in the distance covered daily on foot, the degree of GP Ib expression decreased, thereby providing conditions for the weakening of platelet adhesion.

The results of this research and some earlier study by other authors suggest that the associated levels of GP Ib and GP IIb-IIIa molecules expression on platelets are associated with the level of daily physical activity. In case it increases regularly, there is a decrease in the number of these glycoproteins on the platelet surface. Besides, in those males who increased their physical activity, there were noted direct correlations between the level of GP Ib and GP IIb-IIIa and the mean platelet volume. This can be explained by the strong dependence of the decrease in the number of these receptors on platelet membranes from the decrease in the surface area of each platelet under conditions of regular physical activity. From the sources available, we learn about the relationship between the activity of the collagen receptors expression, which is important for adhesion on platelet membranes, and the intensity of environmental effects on the body (Best *et al.*, 2003; Huang & Sahud, 2003). It is recognized that an essential regulator of platelet activity, keeping it at the optimum level, is an increase in daily physical activity (Mikhaylova *et al.*, 2021). An increase in the distance covered on foot a day contributes to a decrease in the size of the main part of platelets in the peripheral blood and a weakening of the receptor's expression on their surface. This occurs due to the redistribution of receptors between the surface of platelets and their various internal membranes, including the membranes of platelet granules and the walls of the tubules. In the end, this process weakens the hemostatic properties of platelets (Zavalishina *et al.*, 2021b).

Having assessed the number of adenosine phosphates in the platelets of the examined subjects, there were revealed some differences in this parameter in the groups with an increased level of physical activity and in the group that retained low physical activity. It became possible to find out within our research the differences in platelet levels of ATP and ADP and the degree of their secretion from platelets between untrained and physically active males. A clear selection in the study groups and strict implementation of physical activity in the observation groups helped to establish this. The data was found to indicate the relationship between the level of adenosine phosphate in the platelets of males in the second mature age and the distance they walk a day. As the distance covered on foot a day increased, there was an increase in differences between the average levels of these indicators. Therefore, in the most physically active males from the third study group, the differences with the control, in terms of the ATP and ADP levels in platelets and the degree of their secretion, were the most significant. The decrease in these indicators with increasing physical activity is associated with the dynamics of biochemical processes occurring in megakaryocytes of the bone marrow due to regular muscular exertion (Karpov *et al.*, 2021b; Zavalishina *et al.*, 2021c).

The smallest amount of adenosine phosphates in the composition of platelets and their least pronounced secretion was observed in the most physically active males who made up the third study group. When conducting the study, it became possible to identify in physically active males direct statistically significant correlations between the mean platelet volume and the ATP and ADP content in platelets and with the severity of their secretion. In all the study groups that took up physical training, we managed to find significant, medium-strength correlations for all these

indicators. It was mentioned above that the presence of regular physical activity, without ranking it according to its intensity, reduces the secretion of platelets in adolescence and early adulthood (Savchenko, 2013). The reduction in the ATP and ADP secretion can be explained by a decrease in the number of receptors on the platelet membrane, which inhibits the transmission of the stimulating signal to their cytoplasm as the level of physical activity increases.

Thus, there are reasons to believe that systematic aerobic exercise can reduce the mean platelet volume, and the number of ATP and ADP granules in them, and inhibit their release during aggregation. A decrease in the severity of platelet secretion in the context of an increased level of physical activity largely provides a reduction in the overall hemostatic activity of platelets.

In this regard, we can assume that a significant regulator of platelet secretion activity is the level of daily physical activity. An increase in daily physical activity by an increase in the distance traveled a day contributes to a physiologically beneficial reduction of platelet secretion. The revealed pattern is largely related to the features of the actin and myosin content in the cytoplasm of discoid platelets, activated and aggregating in all the groups of the subjects (Makurina *et al.*, 2022).

Having assessed the actin and myosin concentration in the composition of platelets in the study, we determined its variability in groups with different levels of physical activity. It became possible to find out the differences in platelet concentrations of actin and myosin in the composition of platelet-dissociates; during their activation and platelet aggregation between untrained subjects and physically active subject males of three study groups. The obtained data indicate the relationship between the distance traveled a day and the level of actin and myosin count in platelet-dissociates and the degree of their self-assembly during the development of platelet activation and aggregation in the males of the second mature age. With an increase in the distances covered on foot a day, there was an increase in the differences between the values of these indicators. The lowest actin and myosin content in intact and hemostasis platelets was observed in the males who made up the third study group. As a result, in those males who had the maximum physical activity, the differences with the control, concerning the actin and myosin levels in platelets, were the greatest. The decrease in these parameters with increasing physical activity can be explained by the development of physiological changes in megakaryocytes of the bone marrow in the context of regular physical activity (Zavalishina, 2020; Zavalishina *et al.*, 2021c).

In the study, in physically active and physically inactive men, it was possible to identify direct statistically significant correlations between the average platelet volume and the content of actin and myosin in platelets that are in a state of aggregation in response to their strong and weak inducer. In the intact state and presence of platelet activation in the study groups, we did not manage to find significant correlations for these indicators. From the earlier studies, we learn to know that regular physical activity, unranked by its intensity, reduces the actin and myosin level in platelets in adolescence and early adulthood (Savchenko, 2013; Zavalishina *et*

al., 2018). The reduction in the count of these proteins in platelets can be explained by a decrease in the bulk of platelets formed in the bone marrow, which significantly reduces their capacity. The severity of the molecular mechanisms of platelet formation by megakaryocytes is very strongly associated with the level of daily physical activity (Karpov *et al.*, 2021b). It becomes clear that a very important regulator of the hemostatic capabilities of platelet activity at the stage of their formation is the level of daily physical activity. If it is increased by an increase in the distance of the daily walk, physiologically beneficial changes in the processes of platelet generation develop in the bone marrow. No doubt this is due to the composition of membranes and granules and the protein count in the endoplasmic reticulum of megakaryocytes, which will be transferred to young platelets when they are ligated.

The conducted study was limited by gender (males were examined), by age (subjects of the second mature age were being observed), and by the type of physical activity (only those involved in race walks were examined).

Conclusion

Changes in the aggregation properties of platelets, the average volume of platelets, the content and secretion of adenosine phosphates in them, the level of actin and myosin in them, and the density located on platelet membranes GP IIb-IIIa and GP Ib were assessed. These indicators were determined in males of the second mature age, who increased their physical activity due to the additional coverage of various fixed distances per day. The control group was a group of healthy, physically inactive men of the same age. It was found that with an increase in the length of the daily walking distance, the average volume of platelets decreased, the severity of their aggregation decreased, the number of adenosine phosphates deposited in platelets decreased, the severity of their secretion decreased, the basal content of actin and myosin decreased, their self-assembly was inhibited under conditions of platelet activation and became less GP IIb-IIIa and GP Ib on their surface. According to the results of the study, it became clear that aerobic muscular work performed during the daily coverage of a certain distance can be considered a very significant factor in ensuring the optimum activity of platelets. In this regard, we can claim that daily walking can provide a decrease in the size of the main part of platelets with some inhibition in the process of their cytoskeleton self-assembly and secretory capabilities in the males of the second adulthood; a decrease in the density of the receptors on the surface of their plasma membrane due to the transfer of a certain number of them on the inner membranes with a decrease in their ability to aggregate. The obtained results not only complement the existing knowledge on platelet physiology but also give us the possibility to regulate platelet activity in modern clinically healthy males using non-drug treatment. The data from the study performed can be taken into account when designing various clinical recommendations for a therapeutic, neurological, and cardiological profile.

Acknowledgments: The team of authors thanks the administration of the Russian State Social University for the opportunity to research its basis.

Conflict of interest: None

Financial support: None

Ethics statement: The study was approved by the local ethics committee of the Russian State Social University on September 15, 2018 (protocol №11).

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