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Original Article

Impact of physical effort on von Willebrand factor's activity and concentration in blood

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Abstract
Background and Study Aim: Physical exercise causes a wide range of physiological changes including the production and release of haemostasis regulating factors. One of these factors is von Willebrand factor (vWf)- a glycoprotein responsible for both platelet adhesion and coagulation cascade.
Material and Methods: The impact of physical effort on von Willebrand's factor's concentration (vWf:Ag) and activity (vWf:CBA) underwent analysis in the group of 42 people (22 men and 20 women) aged 19-22 (mean 20, SD 8 months). Physical effort was induced by 35 minutes of swimming in indoor swimming pool.
Results: Physical exercise in form of swimming causes an increase of Willebrand's factor's concentration in the blood. Effects size noted in the above studied sample was large. Moreover, interaction of sex and physical exercise seems to be a significant factor in Willebrand's factor's concentration, which seems to be lower in males after physical exercise test in form of swimming.
Conclusions: Even a moderate exercise significantly increases vWf levels and changes coagulation parameters.
Key words*:* von Willebrand factor, primary and secondary haemostasis, fibrinolysis, moderate exercise, indoor swimming.

Glossary. *Abbreviations*: vWF- von Willebrand factor; vWf:Ag- von Willebrand factor activity; vWf:CBA- von Willebrand factor concentration; RCof- ristocetin cofactor; GPIb- glycoprotein Ib; GPV- glycoprotein V; GPIX- glycoprotein IX; HR- heart rate; ELISA- enzyme-linked immunosorbent assay; aPTT- activated partial thromboplastin time; PT- prothrombin time; FVIIIC- coagulation factor VIII; TAT- thrombin-antithrombin complex; PAI-1- tissue type plasminogen activator inhibitor/

Introduction

Physical effort causes a wide range of physiological changes in organism, including the synthesis of proteins responsible for haemostasis. One of such proteins is von Willebrand's factor (vWf). It is glycoprotein with molecular mass up to 20 x 10^6 D produced by vascular endothelial cells, as well as by subendothelial connective tissue and α-granules of platelets. vWf's functions depend on primary and secondary hemostasis. The damage of blood vessels' endothelium initiates both primary and secondary haemostasis. Exposition of platelets to subendothelial matrix begins the adhesion and activation of platelets. Adhesive interactions require multiple receptors on the surface of platelets. These receptors are targeted by adhesive proteins, including Von Willebrand factor (Gale, 2011; Sadler, 1991). vWf attaches to platelets via GPIb/GPV/GPIX receptors and "anchors" the complex to the side of damage. Platelets-vWf-subendothelial matrix complex activates platelets, initiating platelets aggregation. The activation of platelets exposes surface receptor to ligands including fibrinogen, vWf, collagen, fibronectin and vitronectin (Varga-Szabo, Pleines, & Nieswandt, 2008). Next, so called bridges are formed and propagation of the platelet plug begins. The primary platelet plug is instable and requires the formation of insoluble, cross-linked fibrin by coagulation cascade. The exposure of blood components to collagen in subendothelial matrix activates factor XII, that in turn initiates coagulation (both intrinsic and extrinsic pathways). Factors XII, XI, IX, X and VIII participate in prothrombin conversion to cross-linked fibrin. Factor VIII activity is blocked by vWf. The dissociation of vWf from factor VIII converts it to the active form VIIIa. Factor VIIIa is a co-factor for proteolytic activation of factor X by factor IXa, in the Ca^{2+} and phospholipids dependent manner. Factor Xa is a start point for final common pathway or resulting in fibrin synthesis (Bhopale & Nanda, 2003; Fang, Wang, & Wang, 2007). Taken together, von Willebrand's factor participates in platelets aggregation during primary haemostasis and its dissociation from factor VIII allows fibrin formation.

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Due to its role in primary and secondary haemostasis, either vWf concentration (vWf:Ag) or activity (RCof, vWf:CBA) disturbances, therefore, have clinical implications, including life threatening conditions such as thrombosis, arterial thrombosis, congenital or acquired bleeding disorders, for example the von Willebrand's disease, thrombotic throbopenic purpura (Spiel, Gilbert, & Jilma, 2008; Van Schie, Van Loon, De Maat, M., & Leebeek, 2011; Ginsburg et al., 1992; Cruz, Whitelock, & Dong, 2003; Favaloro, 2000; Martinelli, 2005). Assessing individual risk profile, factors influencing vWf:Ag and vWf:CBA must be taken into account. These factors includes lifestyle factors, e.g. physical effort, stress, environmental factors and genetic factors associated with circulation, e.g. genetic determinant is ABO blood group (Spiel et al., 2008).

Although physical effort is one of the factors influencing vWf, the impact of exercise on vWf is still far to be well understand. Numerous studies showed that submaximal (70% maximal HR) or maximal (80% maximal HR) exercise increases vWF:Ag and vWf:CBA. This increase is dependent on sex, training routine and fitness score. Less is known about the correlation of vWf and moderate exercise. The results are also limited by small sample size and the influence of other factors, e.g. blood group type, blood vessels constriction or local circulation stasis on the results (Stibbe, 1977; Hansen, Wilsgård, Olsen, & Osterud, 1990; Woodburn, Rumley, Murtagh, & Lowe, 1997; van Loon et al., 1992; Jern, 1989; Jilma et al., 1997; Krzysiek et al., 2001).

Hypothesis

Moderate physical effort causes the increase of the plasma von Willebrand's factor's concentration and activity, thus increasing the probability of cardiovascular disease.

Purpose

The aim of the research was to evaluate von Willebrand's factor release after moderate exercise. The experimental protocol was designed to reduce the impact of tissue constriction on the release of vWf. Swimming task was chosen as an optimal model of exercise.

Material and methods

Participants

The study was conducted on the group of 42 healthy volunteers swimmers (22 females and 20 males) aged 19-22 (mean 20, SD 8 months). The level of training was similar across the groups. Participants were informed about the study in detail. All participants were told to refrain from strenuous training, food consumption 2 hours before the experiment. Participants were also asked to not to make changes to eating habits and consume alcohol as well.

Procedure

Blood used to determinate vWf:Ag concentration and vWf:CBA activity was drawn approximately 10 minutes before a 35-minute period of freestyle swimming and immediately afterwards. Blood was drawn in accordance to standard procedure from cubital vein, using sodium citrate in 1:9 ratio as anticoagulant, centrifuged for obtaining platelet pour plasma, which was then frozen and stored until the examination date in refrigerator at the temperature -20º C. Von Willebrand's factor's concentation (vWf:Ag) was determined using enzyme-linked immunosorbent assay (ELISA) with the application of commercial DAKO (Denmark) reagents according to the company recommended procedure. The measurements of vWf's activity were performed using the test of binding to collagen according to Falvarolo procedure (vWf:CBA) with modifications of the flattening process (using Sigma, USA, human collagen type III and DAKO conjugate) (Favaloro, 2000; Paczuski, 2002). The experiment was carried out upon the approval of the committee of bioethics, as well as written approval of a subject. The project was approved by University of Nicolaus Copernicus Bioetics Committee and followed by confirmed agreement of all participants according to required procedure.

Statistical analysis.

Shapiro-Wilk test was used to examine the normality assumption. Levene's test was used to test homogeneity assumption. While normality assumption was not met, Wilcoxon test was used to compare variables before vs after swimming using statistical package STATISTICA 13.1 (StatSoft, Inc.). Effect size was calculated using the following formula:

$$r = z / N^{-1/2}$$

Where Z is the z-score from applied nonparametric test and N is the total number of observations. Data was visualized using R with ggstatsplot package. Two-way ANOVA with group factor (male/female) and time (before/after) assumptions were met were analyzed using R package for Aligned Rank Transform for Nonparametric Factorial ANOVAs (type III test) Post-hoc test was done using contrasts (F-M: before-after) using phia R Shape of violin figures indicates distribution and minimal and max observed values. Red dot connected by line indicates mean value, horizontal black line inside the box indicates median value. Mean value and confidence interval (- 95 %; 95 %) are reported in text and alpha level set on 0.05.

Results

The obtained results show that the moderate exercise causes the increase of the plasma von Willebrand's factor's concentration and activity both in men and women. Both measured parameters: vWf:Ag and vWf:CBA increased respectively from 98,67% and 97,21% before the exercise to 156,26% and 165,45% after swimming (p<0,001) (Figure 1, Figure 2).

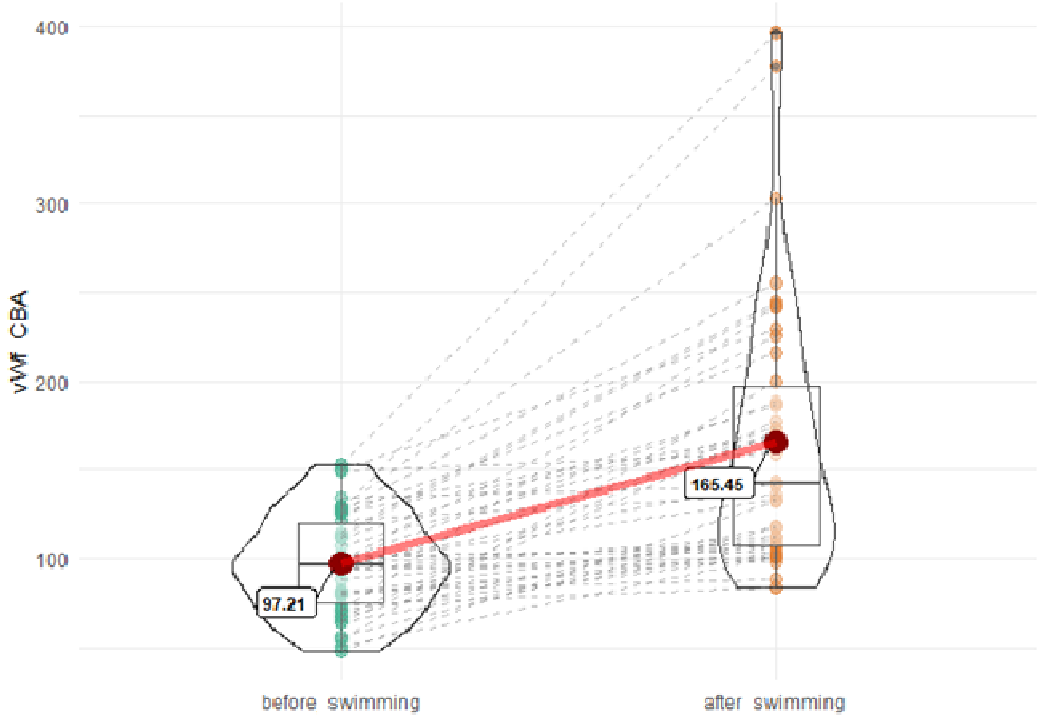

Figure 1. Effects of physical exercise on vWf: CBA

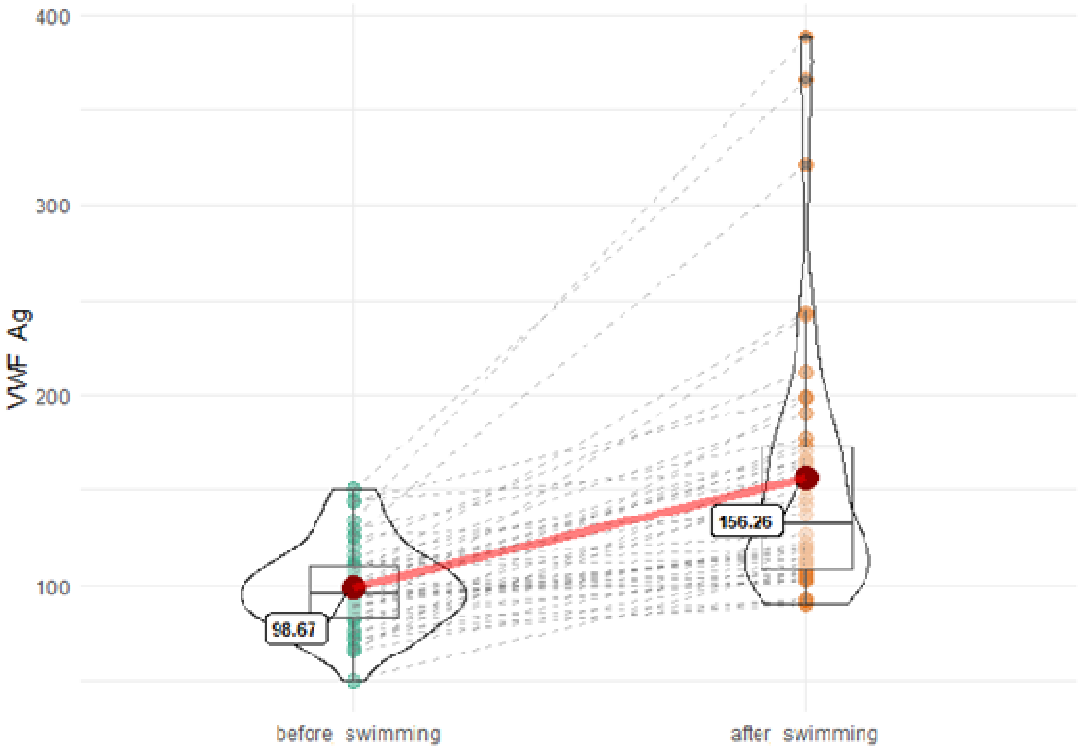

Figure 2. Effects of physical exercise on vWf:Ag

In male group, the mean vWf:CBA % increased from 98,55% (87,23%, 109,27%) to 133,55% (111,89%, 155,21%) (before vs. after exercise, p<0,001). The mean vWf:Ag was increased as well. vWf:Ag before the swimming was 91,75% (83,32%, 100,18%) and 119,95 (108,26%, 131,64%) after the swimming (p<0,001) (Figure 2, Figure 3). In female group vWf:Ag and vWf:CBA changes were higher compared to males (p<0.001). The vWf:CBA before and after the exercise was 96,27% (82,02%, 110,52%) and 194,45% (157,49%, 231,42%) respectively. vWf:Ag change from 104,95% (93,61%, 116, 3%) to 189,27% (153,54%, 225,00%) after the exercise (Figure 3, Figure 4).

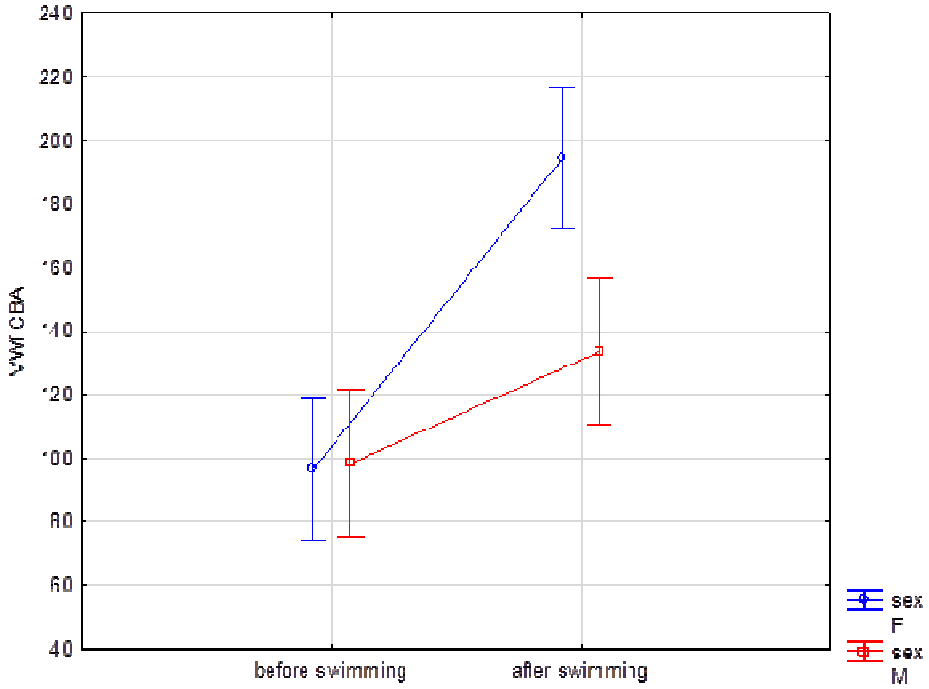

Figure 3. Effects of physical exercise and sex on vWf:CBA

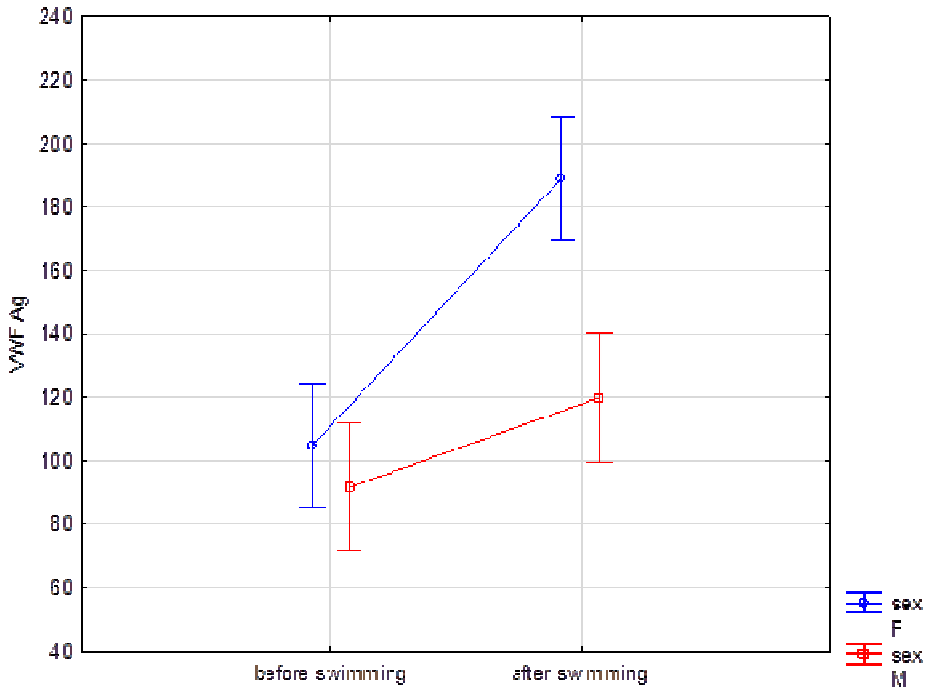

Figure 4. Effects of physical exercise and sex on vWf:Ag

The effects of sex, time and interaction of these two factors were statistically significant (Table 1, Table 2).

Factor	F value	p
sex	21.95	0.00001
time	50.71	0.0000000004
Sex*time	10.31	0.002

Table 1. Effects of physical exercise and sex on vWf:CBA

Factor	F value	p
sex	8.29	0.005
time	51.02	0.0000000004
sex*time	9.32	0.003

Table 2. Effects of physical exercise and sex on VWf:Ag

Discussion

In all examined volunteers, the exercise consisting in a 35-minute swimming, caused the increase in vWf:Ag and vWf:CBA in blood. Significant statistic differences were observed between vWf:Ag and vWf:CBA and sex. Men responded to moderate exercise with the lower increase of vWf:AG and vWf:CBA compared to women. Our result are in line with several studies showing that moderate to maximal exercise increases vWf:Ag and vWf:CBA both in untrained humans and animals (Stibbe,; 1977; van Loon et al., 1992;). Jern et al., 1989; Jilma et al., 1997; Krzysiek et al., 2001; Sano, Motomiya, & Yamazaki, 1980; Musumeci et al., 1989; Small et al., 1984; van den Burg et al., 1995; van Mourik et al., 1999; Vicente, Alberca, & Mannucci, 1984; Ribeiro et al., 2008; Gonzales, Thistlethwaite, Thompson, & Scheuermann, 2009). Increased platelet counts, activated partial thromboplastin time (aPTT), prothrombin time (PT), clotting activity of factor VIII (FVIIIC), von Willebrant factor (vWf), fibrinogen and thrombin-antithrombin complex (TAT) concentration were increased. D-dimer and tissue-type plasminogen activator concentration (t-PA) were elevated as well. In contrast, the concentration of plasminogen activator inhibitor 1 (PAI-1) was decrease (El-Sayed, Sale, Jones, & Chester, 2000; Wang & Liao, 2004; Ribeiro et al., 2007; van Loon et al., 2014). The similar pattern of changes was observed in high fitness level subjects, confirming our results regarding vWf:Ag and vWf:CBA alternations (Rock, Tittley, & Pipe, 1997; Huskens et al., 2016.

In the experiment we also evaluated the impact of sex on vWf:Ag and vWf:CBA after indoor swimming in high fitness activity subjects. Our results showed that sex exerted profound effect on vWf. Men and woman had similar concentration and activity of vWf prior the exercise. The lower increase of both vWf:Ag and vWf:CBA was observed in male group compared to females in response to the indoor swimming. These results contrasts with results obtained by van Loon et.al. They showed that vWf:Ag and vWf:CBA changes were higher in male group after the exercise. It must be noted that the experiment was conducted on low fitness activity level subjects (van Loon et al., 2014). Thus the comparison of our and van Loon et.al results is impeded. However, differences between vWf:Ag and vWf:CBA changes post exercise and sex cannot be undiscussed. Several other studies examined the changes of vWf:Ag and vWf:CBA in mixed group (men and females) but the authors did not analyze the effect of sex on vWf,:Ag and vWf:CBA (Krzysiek et al., 2001; Small et al., 1984; van den Burg et al., 1995; van Mourik et al., 1999; Gonzales et al., 2009; Paton et al., 2004).

Although our experiment focused on vWf:Ag and vWf:CBA measurement, the changes of vWf concentration and activity are not sufficient to determine the influence of exercise on primary and secondary haemostasis or fibrinolysis. Several experiments revealed that fibrinolysis was superior over primary and secondary haemostasis. The rise of concentration of factors associated with primary and secondary haemostasis, e.g. platelet counts, aPTT, PTT, vWf, TAT, FVIII C may indicate the increased rate of primary and secondary haemostasis. Moreover, it may suggest the direct correlation between moderate to maximal exercise and increased risk of cardiovascular system diseases. However, increased D-dimer and t-PA concentration together with decreased PAI-1 concentration show enhanced fibrinolysis.

Not only sex but also other factors, not examined in our experiment, have a great impact on vWf:Ag and vWf:CBA changes after the exercise. These includes: the type and duration of exercise, fitness activity level, age and blood group type of the participants. According to several reports the increased duration of exercise is followed by the proportional increase of vWf:Ag and vWf:CBA (van den Burg et al., 1995; van Loon et al., 2004; Andrew, Carter, O'Brodovich, & Heigenhauser, 1986. Furthermore, the fitness activity level influences the performance of exercise, thus effecting exercise duration during exercise to exhaustion (van Loon et al., 2014). This observation is limited to low fitness activity level persons without the regular fitness physical

activity. Repeated exposure to the strenuous exercise during strength/conditioning training program may exert effect on coagulation and fibrinolysis. In line with this, van der Vorm et.al reported that repetitive submaximal intensity cycling decreased FVIII and vWf concentration on subsequent days of the training (van der Vorm et al., 2018). The results were not confirmed by El-Sayed et.al. The authors observed no changes in APTT, thrombin clotting time, factor VIII procoagulant time and factor VIII antigen concentration. Thus there were no differences in coagulation and fibrinolysis in response to maximal exercise before and after the training program (El-Sayed, Lin, & Rattu, 1995). The observation by El-Sayed et.al is difficult to explain, in our opinion. Simultaneous increase of coagulation and fibrinolysis has protective function. As mentioned, strenuous exercise elicits transient increase of the primary and secondary haemostasis elements, and the cardiovascular disease risk should be increased in high fitness level subjects. However, the enhanced fibrinolysis rebalances primary and secondary haemostasis and decreases the cardiovascular disease risk, acting as cardiovascular protective mechanism.

Participants age is significant as well. Riberio et.al showed differences between 10 years old boys and adult men in coagulation and fibrinolysis pattern (Ribeiro et al., 2007). These data are further supported by Menzelen and Hilberg results. The comparison of coagulation and fibrinolysis pattern in young adults and middle aged persons reveled differences among two groups. The exercise-induced primary and secondary haemostasis changes were enhanced in the middle-aged participants in comparison with younger participants (Menzel & Hilberg, 2009).

We also postulate that fitness physical testing protocol should be individualized according to the type of participant being tested. Factor such as participant's familiarization with the exercise must be considered as important (Evans & White, 2010).

Conclusions

In conclusion, our results show that submaximal exercise of participants with high fitness level influences coagulation. However the results are not sufficient to evaluate primary and secondary haemostasis or the balance between coagulation and fibrinolysis. Numerus studies confirmed the changes of primary and secondary haemostasis as well as fibrinolysis after the exercise. However, the exact pattern of changes is not clear. Therefore additional study must be conducted to investigate the influence of indoor swimming on fibrinolysis. The impact of exercise type and duration, fitness activity level, sex and blood type group of the participant on fibrinolysis should be examined as well.

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