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# Early response of selected haemostatic and haematological parameters to physical activity in young women – the potential impact of oral contraceptives

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## Abstract

**Introduction and objective.** Exercise (submaximal) in untrained subjects can modify haemostasis toward hypercoagulability, especially among women using oral contraceptives (OC). The aim of this study was to investigate whether this can be explained by platelet haemostasis and changes in the generation of membrane microparticles.

**Materials and method.** Young, healthy women (n=60) were divided into 2 equal groups: a study group OC (+) who had used OC for >3 months, and controls who had never used oral contraceptives OC(-). Exclusion criteria: those with systematic daily physical activity. Participants were subjected to treadmill exercise (Cardiac Diagnostic System; model CH2000) using the Bruce protocol/ AHA guidelines. Platelet aggregation with arachidonic acid (ASPI test) or ADP (ADP test), membrane microparticle (MP) activity, plasma coagulation times (APTT/PT) and blood count were determined before and 45 minutes after exercise.

**Results.** Before exercise, the OC(+) group had slightly higher platelet aggregation (ADP test), significantly lower MP activity, slightly lower PLT and slightly higher PDW rate. Exercise caused slight inhibition of platelet aggregation (ASPI test), and significant decrease in MP activity – only in the OC(-) group. After exercise, in both groups there was a significant decrease in PLT and increase in WBC, more pronounced in OC(+) group.

**Conclusions.** Submaximal exercise was beneficial for haemostasis in women not using hormonal contraception, associated primarily with reduced MP activity. No beneficial effects of physical activity were found for women using hormonal contraceptives, possibly associated with a hypercoagulable state, and higher reactivity of blood platelets under the influence of the use of contraceptives.

## Key words

exercise, contraception, haemostasis, platelet function tests, cell derived microparticles

## INTRODUCTION

Studies on the health promoting effects of exercise have been carried out mostly with regard to its ability to modify risk factors for diseases of the cardiovascular system [1]. Other reasons for conducting studies on exercise have arisen from the present trend, especially among young women, for high physical activity often, without the supervision of professionals. Such apparently superfluous physical activity has been found, in some studies, to adversely affect hemostatic factors modifying these in the direction of thrombosis, e.g. by increasing the adhesion and aggregation of platelets and stimulation of coagulation and fibrinolysis [2, 3, 4]. In contrast, several studies have shown beneficial effects from physical exercise, e.g. by unchanged or even inhibited platelet aggregation [5, 6, 7]. The reasons for these contrasting results are thought to arise from variation in the fitness of the subjects studied or in the amount of physical exercise [8, 3, 9].

Intense physical activity, often defined as 'submaximal' physical activity (with a heart rate 85% of a maximum calculated for a particular age), can be associated with an inflammatory response and the generation/activation of platelets and various cells, including granulocytes, which are the main source for the generation of membrane microparticles with prothrombotic activity [8, 10]. Moreover,it has been demonstrated that platelets and granulocytes, with physical exercise, cause an increase in the expression of tissue factor (TF) in monocytes responsible for the activation of the coagulation process and the generation of thrombin [11]. It appears that the thrombotic action of submaximal exercise, especially in untrained subjects, must be considered part of an inflammatory process. It has been shown in other studies that exercise induces inflammation, reflected by an increase in the concentration of interleukin-6 [10]and a temporary increase in both the platelet (PLT) and leukocyte counts [12, 13].

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Young women taking oral contraceptives (OC) are a group particularly vulnerable to prothrombotic complications resulting from poorly-dosed physical exercise. Research on the prothrombotic properties of OC are very well documented, particularly as a risk factor for venous thrombosis [14, 15, 16, 17].

The mechanisms by which hormonal contraceptives induce hypercoagulability involve the activation of plasma coagulation, increased levels of fibrinogen, the emergence of acquired resistance to activated proteinC [18, 19, 20] and the activation of fibrinolysis [21, 22]. It has been suggested that the phenomenon of increased blood clotting under the influence of hormonal contraception is balanced by the activation of the fibrinolytic system [21], as also suggested by previous studies of the authors of the current study [22]. Among other potential mechanisms of the thrombotic action of hormonal contraceptives, the intensity of the myeloid trombocytopoesis and increased platelet reactivity should be considered. The increase in platelet reactivity under the influence of estradiol is perhaps a consequence of an increase in the percentage of large platelets, which is also reflected in an increase in platelet volume (MPV) [23, 24, 25].

## OBJECTIVE

The presented study investigates whether controlled physical activity in young untrained women, with or without oral contraceptives, modifies haemostasis toward a hypercoagulable state, through the mechanisms of platelet haemostasis and membrane microparticle generation.

This is a pilot study to estimate the number of groups necessary for the assumed significance of the studied effects.

## MATERIAL AND METHODS

The study used 60 healthy young women who were students at the Pomeranian Medical University in Szczecin, ranging in age from 21–26 years, who were divided into 2 groups: the test group,who had been using oral contraceptives (mostly generation III OC) for at least 3 months [OC(+); n=30, age 24.1±3.8 years] and a control group, who reported never to have used OC [OC(-); n=30, age 23.9±3.5 years]. Both groups had normal and non-statistically different body mass indices (BMI; 21.2±1.9 kg/m$^2$ and 21.9±3.4 kg/m$^2$, respectively). Exclusion criteria were: venous thrombotic disease, history of cardiovascular disease, tobacco smoking, and women with regular daily physical activity.

Participants were subjected to treadmill exercise (Cardiac Diagnostic System; model CH2000, Cambrige Heart, Inc.,) using the Bruce protocol and AHA guidelines. The test was conducted in the Laboratory for Stress Testing at the Pomeranian Medical University Cardiology Clinic. Resting electrocardiogram (ECG) and resting blood pressure (BP) were registered before the test. If the participant exceeded their submaximal heart rate according to their age, or there were symptoms of exercise intolerance, the test was terminated. Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and metabolic equivalent of task (MET) were analyzed during the test: MET determines the quantity of work completed during the test was calculated using the Blair equation:

MET=[(1.67×V)+(0.3×V×I)+3.5] /3.5 where V=speed of the treadmill in km/h, I=incline of treadmill (%).

The mean metabolic cost of the exercise equalled 9.7 MET which corresponds to heavy exercise [26]. The Ethics Committee at the Pomeranian Medical University in Szczecin approved all the methods and procedures of this study. All participants were informed of the aims and nature of the study and provided written consent to participate.

**Laboratory analyses.** For the assessment of the hypercoagulable state, measurements of platelet aggregation, membrane microparticles and plasma clotting times, i.e. activated partial thromboplastin time (APTT) and prothrombin time (PT) were used. Venous blood was drawn twice: firstly after fasting and before exercise, and secondly 45 minutes after the end of the physical exercise.Blood before and after exercise was collected as 3 samples, each with different anticoagulants. Samples numbered 1 contained 5 mL of blood with hirudin for the determination of platelet aggregation in whole blood. Samples numbered. 2 contained 5 mL of blood in 3.2% sodium citrate in a ratio of 9:1. Samples numbered. 2 were centrifuged to obtain platelet poor plasma (15 min, 1,500×g), for determination of the APTT and PT, and platelet poor plasma was further centrifuged to obtain samples of membrane microparticles (MP Activity) (2 min, 13,000×g). Samples numbered 3 contained 2 mL of blood in EDTA for blood morphology assessment (haematological parameters). Platelet aggregation, APTT and PT and blood morphology were assessed using fresh samples of blood/plasma, and membrane microparticle mesurements from plasma samples previously frozen at -80 °C.

Determination of platelet aggregation was carried out by impedance using Multiplate® aggregometry (Dynabyte Medical, Mannheim, Germany), less than 2 hours after blood collection. Two types of assays were used for determination of platelet aggregation: the ASPI test (Roche Diagnostics GmbH, Mannheim, Germany) using arachidonic acid as a platelet agonist, and the ADP test (Roche Diagnostics GmbH, Mannheim, Germany) where the agonist was adenosine 5'-diphosphate (ADP). The extent of platelet aggregation was expressed as the area under the curve (AUC) in arbitrary units AU x minutes.PT and APTT assays were performed using a coagulation analyser (Behring Coagulation Timer; BCT, Siemens, Marburg, Germany), using test kits Pathromtin SL (APTT) and Thromborel (PT) from Siemens. Measurement of membrane microparticles was made using an enzyme immunoassay (Elisa Zymuphen MP-Activity, Hyphen BioMed, 155, rue d'Eragny, F 95000 Neuville-sur-Oise, France).The MP activity results were expressed as phosphatidylserine equivalents (nM).

Blood morphology was determined with a haematology analyzer(ABX Micros 60,Montpellter, France). Parameters assessed were: mean platelet volume (MPV); platelet distribution width (PDW); counts for platelets (PLT), white blood cells (WBC) and red blood cells (RBC); haemoglobin concentration (HGB); haematocrit (HCT); mean corpuscular volume (MCV); red cell distribution width (RDW); mean corpuscular haemoglobin mass (MCH), and the mean corpuscular haemoglobin concentration (MCHC).

**Statistical analyses.** The data were statistically analysed using commercial software (STATISTICA v. 12.0; StatSoft, Tulsa, Oklahoma, USA). After the Kołmogorov-Smirnov

test, a logarithmic transformation was used when necessary (for MP Activity).

Firstly, Student *t*-tests for paired data were used for comparing parameters before and after exercise, and Student *t*-tests for unpaired data for comparing parameters from the OC (+) group with the OC (-) group. Secondly, a two-factor ANOVA for repeated measurements (interaction between oral contraceptionuse and physical exercise) was used with the LSD test as a *post hoc* test. Linear correlations were assessed using Pearson's correlation. A p value of <0.05 was considered significant. Due to the small size of the groups, values with 0.05<p<0.2 as indicative of possible trends were also considered.

## RESULTS

The impact of physical activity on haemostatic and haematological parameters before and after exercise are shown in Table 1 for all of the women tested, regardless of hormonal status. In the results after exercise, compared to before exercise, there was a trend towards an inhibition of platelet aggregation (ASPI assay: 906±225 vs 866±191 AUxmin, respectively; p=0.146), a significant reduction in PLT (256±61 vs 246±61×$10^9$/L; p <0.001), and a decrease in MPV (8.4±0.7 vs 8.3±0.7 fL; p=0.003). After exercise, red blood cell parameters, such as RBC, HGB and HCT, were reduced. In addition, there was a noteworthy significant increase in the number of WBC (6.6±1.6 vs 7.0±1.8 x 10 /L; p=0.005). No further significant changes under the influence of exercise were found (Tab. 1).

Table 2 summarizes the comparison of measured parameters before and after exercise in the 2 groups. Results indicate that hormonal status may affect the post-exercise response as measured by platelet aggregation (ASPI test) and microparticle (MP) level. Compared to before exercise, in the OC(-) group after exercise there was a trend towards inhibition of platelet aggregation (ASPI assay: 899±186 vs 842±161 AUxmin, respectively; p=0.154), and a significant reduction in MP (7.52±6.10 vs 6.06±8.29 nM; p=0.055). These findings were not observed in the OC(+) group. The difference between the 2 groups was confirmed by analysis of variance and LSD (MP: $p^{ANOVA}$=0.05; $p^{LSD}$=0.025) (Fig. 1).

The OC(+) group, compared to the OC(-) group, had slightly higher baseline platelet aggregation (ADP assay: 841±242 vs 760±173 AU x min; p=0.139) with significantly lower MP activity (4.95±4.16 vs 7.52±6.1 nM; p=0.012), slightly lower PLT count (244±49 vs 268±69 x $10^9$/L; p=0.137) and higher PDW (14.3±0.9 vs 13.9±1.3%; p=0.085). The lower baseline MP and higher PDW in the OC(+) group illustrates the effects of hormonal contraception (Fig. 1, 2). In the case of the ADP results and PLT counts, differences between OC(+) and OC(-) persisted also after exertion(ADP assay; 845±164 vs 717±242 AUx min; p=0.012; PLT count 231±51 vs 261±66 x $10^9$/L; p=0.054) (Tab. 2).

In the OC(-) group, a significant reduction was noted in MPV (8.3±0.8 before vs 8.1±0.8 fl after exercise; p=0.003) (Tab. 2), while in the OC(+) group PDW showed a small non-significant reduction (14.3±0.9% before vs 13.9±0.9% after exercise; p=0.165) (Tab. 2). The tendency to decrease in PDW after exercise in the OC(+) group, and more marked decline in MPV in the OC(-) group, illustrates further the interaction between exercise and hormonal contraception

**Table 1.** The impact on physical activity of selected haemostatic and haematological parameters (means ± SD) before and after exercise (both groups together)

| Parameter | Before exercise | After exercise | p |
|---|---|---|---|
| | | | (Paired Student t-test) |
| ASPI test | | | |
| (AU x min) | 906 ± 225 | 866 ± 191 | **0.146** |
| ADP test | | | |
| (AU x min) | 801 ± 213 | 790 ± 199 | 0.391 |
| MP | | | |
| (nM#) | 6.23 ± 5.34 | 5.56 ± 6.22 | 0.527 |
| APTT | | | |
| (s) | 31.6 ± 3.8 | 31.3 ± 4.1 | 0.361 |
| PT | | | |
| (s) | 12.5 ± 1.8 | 12.5 ± 1.6 | 0.445 |
| PLT | | | |
| ($10^9$/l) | 256 ± 61 | 246 ± 61 | **< 0.001** |
| MPV | | | |
| (fl) | 8.4 ± 0.7 | 8.3 ± 0.7 | **0.003** |
| PDW | | | |
| (%) | 14.1 ± 0.9 | 14.0 ± 1.1 | 0.409 |
| WBC | | | |
| ($10^9$/l) | 6.6 ± 1.6 | 7.0 ± 1.8 | **0.005** |
| RBC | | | |
| ($10^{12}$/l) | 4.28 ± 0.34 | 4.20 ± 0.35 | **0.003** |
| HGB | | | |
| (mmol/l) | 7.7 ± 0.6 | 7.6 ± 0.6 | **0.003** |
| HCT | | | |
| (l/l) | 0.36 ± 0.03 | 0.35 ± 0.03 | **0.005** |
| RDW | | | |
| (%) | 12.8 ± 0.8 | 13.0 ± 0.7 | **0.013** |
| MCV | | | |
| (fl) | 85.0 ± 3.8 | 84.9 ± 4.0 | 0.646 |
| MCH | | | |
| (fmol) | 1.79 ± 0.11 | 1.80 ± 0.11 | 0.460 |
| MCHC | | | |
| (mmol/l) | 21.2 ± 1.1 | 21.3 ± 1.2 | 0.267 |

Abbreviations:
AU = Arbitrary Units; nM # = equivalents of phosphatydyloserine

(PDW: $p^{ANOVA}$=0.264, $p^{LSD}$=0.171) (Fig. 2); MPV: $p^{ANOVA}$=0.144, $p^{LSD}$<0.002) (Fig. 3;). Additionally a significant increase in WBC after exercise was found in the OC(+) group (6.6±1.6vs 7.2±1.8×$10^9$/L; p=0.015); in the OC (-) group increase in WBC was significantly weaker (6.6±1.5 vs 6.9±1.8×$10^9$/L; p=0.153) (Tab. 2).

A reduction in PLT occurred in both groups of women, but was more pronounced in the OC(+) group (244±49 before vs 231±51×$10^9$/L after exercise; p<0.001) (Tab. 2) than in the OC(-) group (268±69 before vs 261±66×$10^9$/L after exercise; p=0.058) (Tab. 2), as confirmed by analysis of variance ($p^{ANOVA}$=0.156; $p^{LSD}$<0.001) (Fig. 4).

Table 3 shows the linear correlation coefficients between parameters in both groups together, and in separate OC(+) and OC(-) groups, both before and after exercise. It should be

**Table 2.** The impact of physical activity on selected haemostatic and haematological parameters (means ± SD) in the study OC (+) and control OC (-) group

| Parameter | OC (+) (n=30) | | | OC (-) (n=30) | | |
|---|---|---|---|---|---|---|
| | Before exercise | After exercise | p | Before exercise | After exercise | p |
| ASPI test | | | | | | |
| (AU x min) | 913±219 | 889±260 | 0.556 | 899±186 | 842±161 | **0.154** |
| ADP test | | | | | | |
| (AU x min) | 841[a]±242 | 845[e]±164 | 0.883 | 760±173 | 717±242 | 0.289 |
| MP | | | | | | |
| (nM) | 4.95[b]±4.16 | 5.05±3.07 | 0.887 | 7.52±6.10 | 6.06±8.29 | **0.055** |
| APTT | | | | | | |
| (s) | 31.1±4.3 | 30.8±4.4 | 0.490 | 32.2±3.1 | 31.8±3.7 | 0.520 |
| PT | | | | | | |
| (s) | 12.8±2.4 | 12.8±2.1 | 0.717 | 12.2±0.7 | 12.3±0.8 | 0.408 |
| PLT | | | | | | |
| ($10^9$/l) | 244[c,e]49 | 231[f]±51 | **<0.001** | 268±69 | 261±66 | **0.058** |
| MPV | | | | | | |
| (fl) | 8.5±0.6 | 8.4[g]±0.6 | 0.235 | 8.3±0.8 | 8.1±0.8 | **0.003** |
| PDW | | | | | | |
| (%) | 14.3[d]±0.9 | 13.9±0.9 | **0.165** | 13.9±0.9 | 13.9±1.3 | 0.840 |
| WBC | | | | | | |
| ($10^9$/l) | 6.6±1.6 | 7.2±1.8 | **0.015** | 6.6±1.5 | 6.9±1.8 | **0.153** |

**Abbreviations:** OC(+): taking oral contraceptives; OC (-): no use of oral contraceptives; p: p value from paired Student *t*-test comparing before vs after exercise within the same OC group; index values (a – g) indicate differences between OC groups (unpaired Student *t*-tests): a – d: before OC (+) vs before OC (-) and e – g: after OC (+) vs OC (-); before: [a] p = 0.139, [b] p = 0.012, [c] p = 0.137, [d] p = 0.085; after: [e] p = 0.012, [f] p = 0.054, [g] p = 0.079;
MP (nM): results after logarithmic transformation.

**Table 3.** Coefficients of linear correlation between the studied variables before and after exercise

| Variables | Before exercise | | | After exercise | | |
|---|---|---|---|---|---|---|
| | Whole group | OC (+) | OC (-) | Whole group | OC (+) | OC (-) |
| | **r** | **r** | **r** | **r** | **r** | **r** |
| ASPI: APTT | − 0.25* | − 0.52** | NS | NS | NS | NS |
| ASPI: PT | − 0.30** | − 0.46** | NS | NS | NS | NS |
| ASPI: WBC | + 0.29* | + 0.71*** | + 0.31* | NS | NS | NS |
| ADP: WBC | + 0.49*** | + 0.60*** | + 0.32* | + 0.41*** | + 0.48** | + 0.36 |
| MP: ADP | NS | NS | NS | − 0.27* | NS | − 0.36* |
| MP: APTT | + 0.30** | NS | + 0.38* | NS | + 0.40* | NS |
| MP: PLT | + 0.28* | NS | NS | NS | NS | NS |
| PDW: APTT | − 0.39** | − 0.54** | NS | NS | NS | NS |
| PDW: PT | − 0.29* | − 0.49** | NS | NS | NS | NS |
| PDW: PLT | − 0.29* | − 0.33* | NS | NS | NS | NS |
| PDW: MPV | + 0.69*** | + 0.64*** | + 0.72*** | + 0.57*** | + 0.41* | + 0.68*** |
| PLT: MPV | − 0.47*** | − 0.54** | − 0.41* | − 0.39** | − 0.49** | − 0.24* |
| PLT: WBC | + 0.33** | + 0.31* | + 0.36* | NS | NS | NS |

**Abbreviations:** OC(+): taking oral contraceptives; OC (-): no use oral contraceptives;
* p < 0.05; ** p < 0.01; *** p < 0.001 from Pearson's correlation test

noted that significant correlations for both groups together were found, after further analysis, to be mostly due to the OC(+) group. The most interesting correlations in the OC(+) group before exercise included negative correlations between

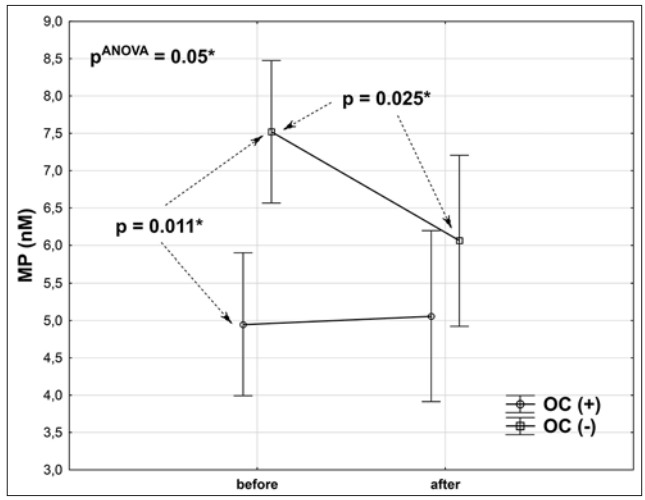


**Figure 1.** The impact of physical activity and oral contraceptives on membrane microparticle activity (MP)
Abbreviations:
* statistical significance after logarithmic transformation

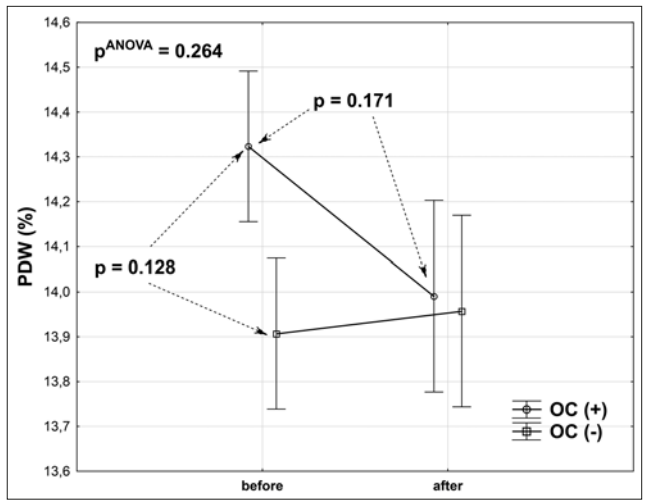


**Figure 2.** The impact of physical activity and oral contraceptives on the variability of platelet volume (PDW)
Abbreviations:
PDW: Platelet Distribution Width

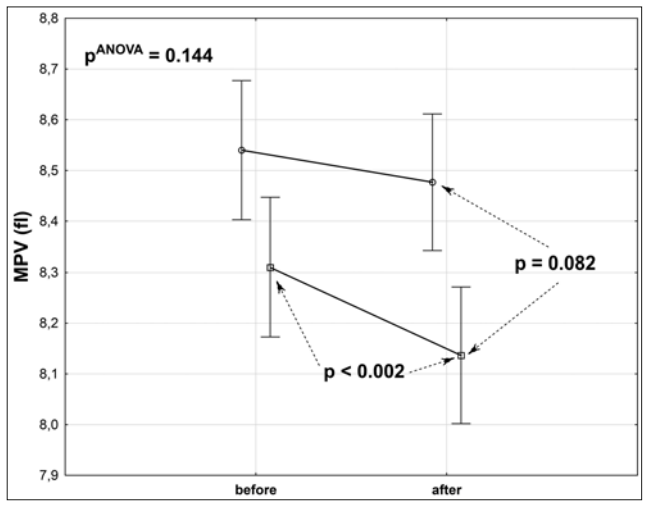


**Figure 3.** The impact of physical activity and oral contraceptives on mean platelet volume (MPV)

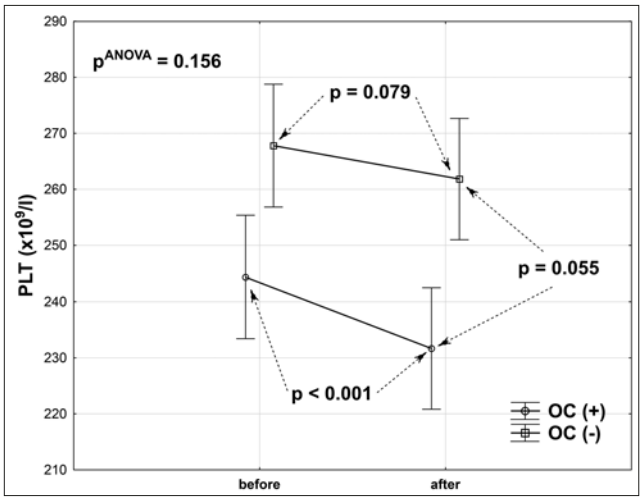


**Figure 4.** The impact of physical activity and oral contraceptives on platelet count (PLT)
Abbreviations for Figs. 1,2,3,4
$P^{ANOVA}$: two-factor ANOVA for the determination of the interaction between physical activity and oral contraceptives
p: *post hoc* LSD test for comparison between groups
OC (+): taking oral contraceptives
OC (-): no use of oral contraceptives

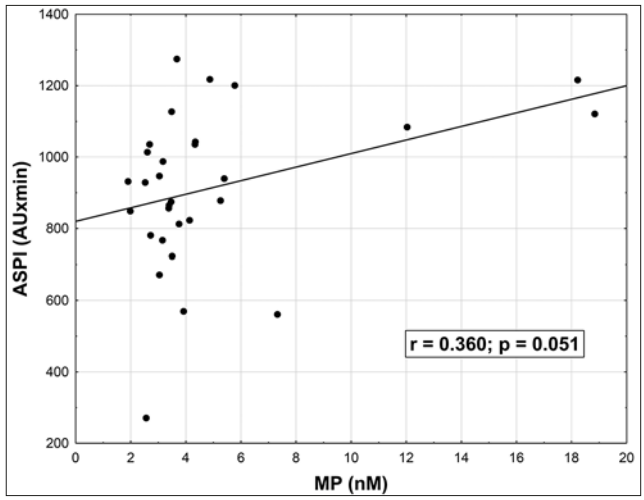


**Figure 5.** Pearson correlation coefficients between platelet aggregation induced by arachidonic acid (ASPI test) and microparticle activity (MP) in OC (+) group before exercise

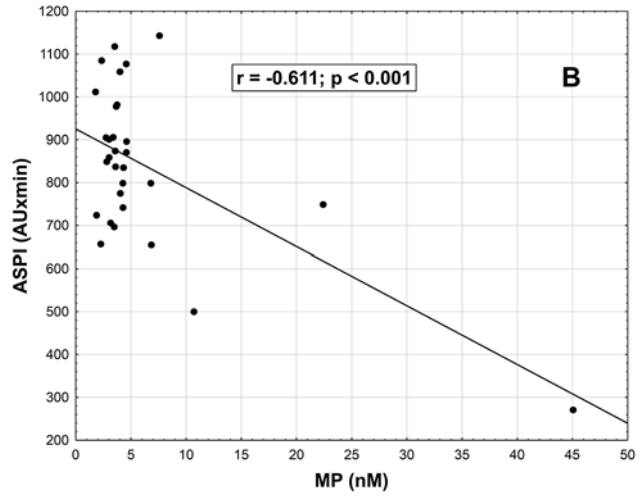


**Figure 6.** Pearson correlation coefficients between platelet aggregation induced by arachidonic acid (ASPI test) and microparticle activity (MP) in OC (-) group after exercise
Abbreviations for Figs 5, 6
OC (+): taking oral contraceptives; OC (-): no use of oral contraceptives

aggregation in the ASPI assay with the coagulation times APTT (r=-0.52) and PT (r=-0.46), and positive correlations between aggregation in both assays (ASPI and ADP) with the WBC count. Although correlations between ASPI, or ADP, and WBC were found in both groups, the correlations in the OC(+) group had a greater slope (r=0.71; r=0.60) than in the OC(-)group (r=0.31; r=0.32). All other significant correlations occurred only in the OC(+) group: negative correlations were found between PDW and the 3 parameters APTT, PT and PLT.

It should be noted that the correlations found before exercise did not occur after exercise, except for a persistant correlation between the ADP assay results and WBC count; after exercise this was stronger in the OC(+) group than in the OC(-) group (r=0.48; p < 0.01; r=0.36). A positive correlation was detected between PDW and MPV and a negative correlation between PLT and MPV; these correlations were observed in both groups (OC(+) and OC(-), both before and after exercise.

A positive correlation was found between ASPI assay results and MP in the OC(+) group before exercise (r=0.36) (Fig. 5), and a negative correlation between ASPI assay results and MP in theOC(-) group after exercise (r=-0.61) (Fig. 6).

## DISCUSSION

The results showed that submaximal exercise modified the processes of platelet aggregation and the activity of membrane microparticles in a manner dependent on the hormonal status of the women. Physical activity favourably influenced the processes of platelet haemostasis, slightly reducing platelet aggregation induced by arachidonic acid (in the ASPI assay) in the whole group of women, possibly only in those who had reportedly never used oral contraceptives. Although most studies have shown that the activation of platelets and coagulation processes occur under the influence of exercise [27, 28, 29]. Some researchers have reported a lack of effect [5, 6], or even an inhibitory effect in the processes of blood platelet aggregation [7]. One reason for these varying results is possibly the amount of training the subjects had received. Some studies have suggested that submaximal exercise in untrained subjects generally had a negative impact, including the activation of blood coagulation [8].

In the current study, both groups of women OC(+) and OC(-) were untrained, this is therefore a possible cause of the different response of platelets to exercise was their hormonal status. It seems that the lack of a favourable inhibitory effect of physical activity on blood platelet aggregation in the ASPI assay, especially in OC(+) group, could be due to higher platelet reactivity at baseline, i.e. before exercise, which was manifested in increased, compared to the OC(-) group, platelet aggregation in the ADP assay. According to some researchers, increased platelet reactivity in women taking oral contraceptives may be caused by an increase in the percentage of large platelets, resulting from accelerated turnover of platelets [23, 24, 25]. In the presented study, increased turnover of platelets in women taking OC is shown by, firstly, the increased platelet distribution width (PDW) and a slightly lower number of platelets [in

comparison with the OC(-) group], and secondly, by a strong positive correlation between the PDW and platelet volume (MPV) and a negative correlation between MPV and the PLT count. The relationship between the volume of blood platelets with the PLT number is clearly shown in the literature, as is the association between an increase in the percentage of large platelets and a reduction in the number of platelets [30]. Although these correlations in this study also occur in women without OC, they are weaker, and it must be emphasized that they may be of particular importance for women using hormone therapy with estrogen composition.

This is especially important due to the fact that an acceleration in megakaryocytopoiesis can be due to response via the estrogen receptor present in megakaryocytes and platelets, resulting in an increase in the percentage of large platelets [31, 32]. According to several studies, large platelets, compared with small platelets, are more reactive metabolically and enzymatically and produce more thromboxane TXA2, which is associated with an increased reactivity of platelets to platelet agonists and activation of coagulation [33]. The association of large platelets with the activation of blood coagulation in the current study was indicated by a strong negative correlation between PDW and APTT/PT clotting times, detected only in the OC(+) group.

Indirect evidence of increased platelet reactivity and simultaneous activation of coagulation while taking OC, may also be indicated by the significant negative correlation between the ASPI aggregation assay results and the APTT and PT coagulation times, which was only detected in the OC(+) group. This interpretation is consistent with literature reports showing the effects on many of the coagulation processes under the influence of oral contraceptives [19, 34, 35]. It is worth noting that increased platelet activation and, therefore, an increased risk of thrombosis while taking hormonal contraceptives, might be connected with a reduction in the concentration of nitrites/nitrates in blood plasma, which has been demonstrated in long-term studies in pre-menopausal women [36]. Recent research on the mechanisms of thrombotic action of exogenous female hormones, indicate that risk factors such as smoking and inflammation significantly increases the risk of blood clots while using these hormones [36, 37].

This study has demonstrated a moderate association between platelet activation and inflammatory processes by a strong positive correlation of platelet aggregation (as measured by both assays, but especially with the ASPI test), with the number of leukocytes detected only in women taking OC.

The increase in platelet aggregation, together with an increase in white blood cell count, may be largely explained by lack of inhibition of platelet aggregation under the influence of physical activity in OC(+) women. The presented results have shown that exercise significantly increased the number of leukocytes in the group of women using hormonal contraception.

Some studies have previously revealed that submaximal exercise may induce inflammation, increase white blood cell count, and activate platelets and a variety of cells, including granulocytes, and thus is able to cause an increased generation of membrane microparticles (MP) [8, 10]. In the presented study, exercise slightly inhibited platelet aggregation in the OC(-) group, but there was no effect on aggregation in the OC(+) group, therefore similar behaviour was expected with MP activity.

This study found a significant decrease in MP activity in the OC(-) group, while in the OC(+) group MP activity remained stable, with a slight increasing trend. Thus, the results obtained are not fully confirmed in the literature, since physical exercise generally causes an increase, not a decrease, in the activity of MP. Literature data show that a maximum increase in MP activity occurs 45 minutes after intense physical exercise, and can last up to 2 hours after exercise in trained subjects, or even longer in the case of untrained subjects (hence, in the current study, comparable measurements were made at this time) [3, 8, 10].

Thus, the observed decrease in MP activity here after exertion in the OC(-) group should be considered as a potentially beneficial effect, presumably due to platelet function, indicated by the strong negative correlation between MP and platelet aggregation (from both ASPI and ADP assays). In contrast, no decrease in MP activity during exercise in the OC(+) group could be associated with hormonal status.

Data in the literature indicate that exogenous female hormones significantly increase the MP production from platelets and granulocytes in post-menopausal women [38]. Thus, because of the thrombogenicity of hormonal contraception, it was expected that higher MP activity would be found in the OC(+) group than in the OC(-) group, but in the current study, the reverse was found. It should be noted that the lower MP activity in the OC(+) group, compared to the OC(-) group, may be a random effect associated with the phase of the menstrual cycle in women [39], which was not taken into consideration, and is therefore one limitation to the study. The small sample sizes were a further limitation of the study; however, please note that this is a pilot study and that larger groups are currently under investigation.

Note that the lower MP activity in the OC(+) group, relative to the OC(-) group, may be due to the use of MP in repair processes. There are studies which show a significant role of MP in the process of vascular wall repair [40]. However, it seems that regardless of the basal level of MP activity, membrane microparticles in women using OC are closely associated with platelet aggregation, as indicated by a positive correlation between MP activity and ASPI aggregation, observed only in the OC(+) group before exercise. Given the fact that MP activity, especially platelet-derived, gives an approximately 100-fold higher prothrombotic activity than from activated platelets [41], it should be emphasized that a higher level of MP activity could potentially be a risk factor for thrombosis among women using hormonal contraception. Moreover, this could be the cause of the lack of beneficial effects of physical activity in women taking OC.

The observed data concerning platelet morphology are very interesting in relation to the impact of physical activity. From the literature, an increase in the volume of blood platelets (MPV) has been shown with intense physical activity, confirming the presence of a pool of young active platelets, which could be indirect evidence for the prothrombotic effects of exercise [42]. In this study, in the group of women not using hormonal contraception, the results showed evidence of a significant decline in MPV after exercise.This could be additional evidence for the beneficial effects of exercise, but only among women not using OC.

## CONCLUSIONS

The results of this study suggest that submaximal exercise is beneficial for haemostasis, associated primarily with a reduction in the activity of membrane microparticles, but only in women not using hormonal contraception.

In contrast, the lack of beneficial effects of physical activity among women using hormonal contraceptives may be potentially associated with a hypercoagulable state, and higher reactivity of blood platelets under the influence of hormonal status.

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## REFERENCES

1. Suminski RR, Poston WSC, Foreyt JP, Jeor SS. Physical activity assessed with three different methods and the Framingham Risk Score on 10-year coronary heart disease risk. Med Sci Monit. 2008; 14(1): CR1–9.
2. Hurlen M, Seljeflot I, Arnesen H. Increased platelet aggregability during exercise in patients with previous myocardial infarction. Lack of inhibition by aspirin. Thromb Res. 2000; 99: 487–94.
3. Maruyama K, Kadono T, Morishita E. Plasma levels of platelet derived microparticlesare increased after anaerobic exercise in healthy subjects. J AtherosclerThromb. 2012; 19(6): 585–87.
4. Heber S, Volf I. Effects of physical (in) activity on platelet function. Biomed Res Int. 2015; Article ID 165078:1–11. http:/ /dx.doi.org/10.1155/2015/165078.
5. Lamprecht M, Moussalli G, Ledinski G, Leschnik B, Schlagenhauf A, Koestenberger M, et al. Effects of a single bout of walking exercise on blood coagulation parameters in obese women. J Appl Physiol. 2013; 115(1): 57–63.
6. Cadroy Y, Pillard F, Sakariassen KS, Thalamas C, Boneu B, Riviere D. Strenous but not moderate exercise increases the thrombotic tendency in healthy sedentary male volunteers. J Appl Physiol. 2002; 93(3): 829–33.
7. Aldemir H, Kilic N. The effect of time of day and exercise on platelet functions and platelet-neutrophil aggregates in healthy male subjects. Mol Cell Biochem. 2005; 280(1–2): 119–124.
8. Sossdorf M, Otto GP, Claus RA, Gabriel HHW, Lösche W. Cell-derived microparticles promote coagulation after moderate exercise. Med Sci Sports Exerc. 2011; 43(7): 1169–76.
9. Chen YW, Chen YC, Wang JS. Absolute hypoxic exercise training enhances in vitro thrombin generation by increasing procoagulant platelet-derived microparticles under high shear stress in sedentary men. Clin Sci. 2013; 124(10): 639–49.
10. Chaar V, Romana M, Connes P. Effect of strenuous physical exercise on circulating cell-derived microparticles. Clin HemorheolMicrocirc. 2011; 47(1): 15–25.
11. Lund T, Kvernmo HD, Osterud B.Cellur activation in response to physical exercise: the effect of platelets and granulocytes on monocyte reactivity. Blood Coagul Fibrinolysis. 1998; 9(1): 63–9.
12. Chamberlain KG, Tong M, Penington DG. Properties of the exchangeable splenic platelets released into the circulation during exercise induced thrombocytosis. Am J Hematol. 1990; 34(3): 161–68.
13. Bakovic D, Pivac N, Eterovic D, Breskovic T, Zubin P, Obad A, et al. The effects of low-dose epinephrine infusion on spleen size, central and hepatic circulation platelets. Clin Physiol Funct Imaging. 2013; 33(1): 30–37.
14. Gomes MP, Deitcher SR. Risk of venous thromboembolic disease associated with hormonal contraceptives and hormone replacement therapy: a clinical review. Arch Intern Med. 2004; 164: 1965–976.
15. Vlieg AH, Helmerhorst FM, Vandenbroucke JP, Doggen CJM, Rosendaal FR. The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA Case-control Study. BMJ. 2009; 339: b2921.
16. Lidegaard Ø, Løkkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. BMJ. 2009; 339: b2890.
17. Lidegaard Ø, Nielsen LH, Skovlund CW, Skjeldestad FE, Løkkegaard E. Risk of venous thromboembolism from use of oral contraceptives containing different progestogens and oestrogen doses: Danish cohort study, 2001–9. BMJ. 2011; 343: d6423.
18. Rosendaal FR, Helmerhorst FM, Vandenbroucke JP. Female hormones and thrombosis. Arterioscler Thromb Vasc Biol. 2002; 22: 201–210.
19. Stocco B, Fumagalli HF, Franceschini SA, Martinez EZ, Marzocchi-Machado CM, de Sá MFS, et al. Comparative study of the effects of combined oral contraceptives in hemostatic variables. Medicine. 2015; 94(4): e385.
20. Alhenc-Gelas M, Plu-Bureau G, Guillonneau S, Kirzin JM, Aiach M, Ochat N, et al.Impact of progestagens on activated protein C (APC) resistance among users of oral contraceptives. J Thromb Haemost. 2004; 2: 1594–1600.
21. Weiss C, Walter B, Dorsch MF, Bärtsch P. Fibrinolytic response to exercise in women using third-generation oral contraceptives. Blood Coagul Fibrinolysis. 2006; 17: 563–68.
22. Jastrzębska M, Wajda P, Chełstowski K, Byra E, Sowińska-Przepiera E. Changes in some hemostatic variables during oral contraceptive use in young women; a pilot study. Diagn Lab. 2015; 51(1): 1–14.
23. Negrev N. Female sex hormones and thrombocytopoiesis. Eksp Med Morfol. 1990; 29(2): 57–62.
24. Ranganath LR, Christofides J, Semple MJ. Increased mean platelet volume after oestrogen replacement therapy. Ann Clin Biochem. 1996; 33(6): 555–60.
25. Kaygusuz I, Simavli SA, Eser A, InegölGümüs I, Yüksel S, IltemirDuvan ZC, et al. Effects of oral hormone replacement therapy on mean platelet volume in postmenopausal women. Turk J Med Sci. 2014; 44: 980–84.
26. Pina I, Balady G, Hanson P. Guidelines for clinical exercise testing laboratories. A treatment for Healtcare Professionals From the Committee on Exercise and Cardiac Rehabilitation American Heart Association. Circulation. 1995; 91: 912–21.
27. Hanke AA, Staib A, Görlinger K, Perrey M, Dirkmann D, Kienbaum P. Whole blood coagulation and platelet activation in the athlete: a comparison of marathon, triathlon and long distance cycling. Eur J Med Res. 2010; 15: 59–65.
28. Ribeiro J, Almeida-Diasb A, Ascensaoc A, Magalhaesc J, Oliveiraa AR, Carlsond J, et al. Hemostatic response to acute physical exercise in healthy adolescents. J Sci Med Sport. 2007; 10: 164–69.
29. Kishi Y, Ashikaga T, Numano E. Inhibition of platelet aggregation by prostacyclin is attenuated after exercise in patients with angina pectoris. Am Heart J. 1992; 123(2): 291–97.
30. Bain BJ. Platelet count and platelet size in males and females. Scand J Haematol. 1985; 35: 77–79.
31. Tarantino MD, Kunicki TJ, Nugent DJ. The estrogen receptor is present in human megakariocytes. Ann NY Acad Sci. 1994; 714: 293–96.
32. Nagata Y, Yoshikawa J, Hashimoto A, Yamamoto M, Payne AH, Todokoro K. Proplatelet formation of megakariocytes is triggered by autocrine-synthesized estradiol. Genes & Development, 2003; 17: 2864–869.
33. Köşüş N, Köşüş A, Turhan NO. Ralationship of ovarian volume with mean platelet volume and lipid profile in patients with polycystic ovary syndrome. Exp Ther Med. 2011; 2: 1141–144.
34. Bremme K, Hamad RR, Berg E, Strandberg K, Stenflo J. The APC-PCI concentratuion as an early marker of activation of blood coagulation. A study of women on combined oral contraceptives. Thromb Res. 2012; 130: 636–39.
35. Oslakovic S, Zadro R. Comparison of the impact of four generations of progestins on hemostatic variables. Clin Appl Thromb Hemost. 2014; 20: 448–55.
36. Paluch Z, Jedlicková V. The influence of long-term hormonal contraception and smoking an plasma concentrations of nitrites and nitrates. Med Sci Monit. 2005; 11(2): BR 61–64.
37. Sieminska A, Jassem E. The many faces of tobacco use among women. Med Sci Monit. 2014; 20: 153–62.
38. Rank A, Nieuwland R, Nikolajek K, Rösner S, Wallwiener LM, Hiller E, et al. Hormone replacement therapy leads to increased plasma levels of platelet derived microparticles in postmenopausal women. Arch GynecolObstet, 2012; 285: 1035–1041.
39. Toth B, Nikolajek K, Rank A, Nieuwland R, Lohse P, Pihusch V, et al. Gender specific and menstrual cycle dependent differences in circulating microparticles. Platelets, 2007; 18(7): 515–21.
40. Morel O, Morel N, Freyssinet JM. Toti F. Platelet microparticles and vascular cells interactions: A checkpoint between the haemostatic and thrombotic responses. Platelets. 2008; 19(1): 9–23.
41. Sinauridze EI, Kireev DA, Popenko NY, Pichugin AV, Panteleev MA, Krymskaya OV, et al. Platelet microparticle membrane have 50- to 100-fold higher specific procoagulant activity than activated platelets. Thromb Haemost. 2007; 97(3): 425–34.
42. Lippi G, Salvagno GL, Danese E, Tarperi C, Guidi GC, Schena F. Variation of red blood distribution width and mean platelet volume after moderate endurance exercise. Advances in Hematology. 2014; 2014: Article ID 192173, 4 pages.