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# Effect of short term consumption of cocoa on platelet factors (Plt, MPV, PDW) in male athletes following one session incremental exhaustive exercise

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**Abstract**

**Cardiovascular disease is regarded as the main indicator of cardiac arrests and the leading cause of mortality. Platelets, as the blood factors, play an important role in the etiology of cardiovascular diseases and its associated risk factors. According to the increased risk of abnormal clot formation in main artery by intense exercises and the Platelets role in the promotion of cardiovascular diseases, strategies including cocoa (flavanol-rich foods) consumption has become more widespread. In this semi- experimental study, 20 healthy male soccer players with a mean (22±1years, Vo2max=53.70±1.5) participated randomly in an exhaustive exercise (Bruce) in two consecutive weeks. After the first stage of blood sampling, each athlete consumed around 5 mg kg of the bottles containing placebo or cocoa and two hours following that performed Bruce test. Before, immediately and one hour after the test, blood sampling stages II, III and IV were taken. Data were analyzed by a two- factor analysis of variance (ANOVA) with repeated measures at p<0.01. The results have shown that cocoa consumption solely or in combination with exercise influenced on Plt, MPV and PDW markers and resulted into the decrease of them (P<0.01). And also there was a significant difference between the values of research parameters in stages two and three. These findings indicate that one session of exhaustive aerobic exercise may have an influential effect on blood Plt, MPV, and PDW levels of male soccer players.**

***Key words**:* Cardiovascular disease, platelet, cocoa, male soccer players, Bruce test

## Introduction

Cardiovascular disease is regarded as the main indicator of cardiac arrests and the leading cause of mortality (Willoughby et al., 2002). According to WHO (World Health Organization), the annual number of 7.16 million people worldwide lose their lives due to cardiovascular diseases (Lanza et al., 2003). Various studies on blood have shown its key vital role in cardiovascular diseases (El-sayed, 2005). However, disorder of the natural properties of the blood, as an independent risk factor for coronary heart disease, especially arterial occlusive disease has also been considered. Therefore, abnormality of any of blood factors may indicate an increased risk of different diseases (El-sayed, 2005). Platelets are one of the blood factors, which play an important role in the etiology and pathophysiology of cardiovascular diseases and its associated risk factor (Williams et al., 2006). In recent years, it has been found that platelet plays an important role in pathology of clinical complications and thrombogenesis caused by atherosclerosis and also athrogenesis machanisms (Aruigemma et al., 2007). Although platelets are an important factor in the normal

blood coagulation system, recent evidences demonstrated that abnormal function, aggregation and activation of blood platelets play a pivotal role in acute coronary artery diseases, myocardial infarction, unstable angina and heart attacks (El-sayed, 2005). According to previous studies, investigators have definitely stated that intense exercises can temporarily develop the incidence of initial heart attacks and increase the probability of abnormal thrombogenesis of blood arteries (Singh et al., 2006; Wang et al., 2005; Hilberg et al., 2003, Li et al., 2007) .Intense exercise stimulates pre- thrombosis condition (Li et al., 2007) and probably by this, it can lead to sudden cardiac death and acute myocardial infarction (Douglas et al., 1998). On the other hand, intense exercise increases platelets function (activity, aggregation capability, activation and adhesion of platelets) (El-sayed, 2005; Singh et al., 2006; Ahmadizad et al., 2006). Sinzinger et al, (1988) stated that sudden heart failure and obstruction caused by blood coagulation among athletes during intense exercises may be due to high function of platelets. Li et al (2007), shown that exhaustive intense exercise on treadmill results into the increase of platelet number in healthy males. In a study by Prasa et al (2003), they concluded that extreme short- term exercise causes the abnormal blood coagulation, so that fibrinolysis clearly increases and this increase is directly related to exercise intensity. Hansen et al (1990) stated that after intense physical exercise an athlete is more susceptible to complications resulted from blood coagulation in arteries. Weiss et al (1998) proclaimed that the risk of blood coagulation (increased activity of platelets and high coagulation mode of blood) occurs after heavy exercise (80% VO2max) in healthy male subjects. Given the mentioned issues and cardiovascular disease as the main cause of mortality in the present century and also the essential role of platelets in the formation and promotion of cardiovascular diseases such as acute myocardial infarction, heart attacks and venous thromboembolism, anti- platelets strategies have been developed in order to the prevention of these complications and also its secondary occurrences (Keen et al., 2003; Lars, 2005). Cocoa, because of its polyphenols (nutrients with anti – oxidative properties) and therefore, its antioxidant properties, modulates the activation and function of platelets and also blood homeostasis, and decreases the risk of abnormal blood coagulation formation. Epidemiology studies have demonstrated an inverse relation between mortality by cardiovascular diseases and by polyphenol (Cooper et al., 2007; Schroeter et al., 2006). Through the prevention of platelets function and aggregation, Cocoa modulates their function and protects the subjects against cardiovascular diseases (Pagolieroni et al, 2000). Mechanisms related to biological effects of foods containing polyphenols such as Cocoa, including anti- oxidant effects, involve the ability to modulate the gene expression and specific cellular messaging pathway's and also the ability to influence on cell membrane properties and the function of cellular receptors (Keen., 2005; Sies et al., 2005).

However, all studies on cocoa consumption have focused on anti-oxidant activity and oxidative stress, vascular expansion and endothelial function, immune function and anti- inflammatory effects and finally the different functions of platelets. For instance, Rein et al, (2000) in a study on the effect of cocoa on the function and activation of platelets have concluded that cocoa consumption inhibits platelets activation and function and has an effect similar to aspirin on homeostasis. Murphy et al, (2003) also shown that cocoa consumption in healthy subjects causes a significant decrease in aggregation and activation of platelets. However Singh et al (2006), stated that a physical exercise by 70%VO2max intensity in combination with cocoa consumption has a very small effect on platelet function in response to exercise .Blood platelets and coagulation factors have an important role in the formation of abnormal thrombus, initial atherosclerotic lesions and cardiovascular diseases (Rein et al., 2000). Therefore, in order to determine the specific dietary compounds and effective drugs and the process of their impact, and also the clinical importance of these findings in relation to thrombosis, atherosclerosis and cardiovascular diseases during exercise and physical activity, more researches will be needed (Pagolieroni et al., 2000). So, due to the importance of cardiovascular system and blood in human body and given the rare research findings on this issue(no research on cocoa and platelets) and researchers' recommendations and also the focus of most of these researches on patients or non- athlete healthy subjects, the present study was done by the purpose of the influence of short- term cocoa consumption with combination of one session incremental exhaustive exercise on platelet factors (platelet count,platelet volume and platelet distribution).In other words, the main question of this study is that whether cocoa with a specific dosage(consumption rate) can inhibit the increase of platelet coagulation factor following an exhaustive exercise or even reduce its rate to some extent or not?

## Materials and Methods

### Statistical Population and Sample

The present research is a 2× 4 factorial design repeated measurement study with control group (one group) and double- blind, was done on 20 young soccer players with mean (age 22± 1, fat percent 22.50± 1.2 % and VO2max = 53.70 ± 1.5 ml/kg.min). Subcutaneous fat thickness of subjects by Lafayette caliper with 1127 models manufactured in the USA in three-point method (brachial triceps, abdominal and pelvic above) was measured. All measurements in two innings, the right side of body and within 20 seconds between appointments were performed. Average was recorded twice. To calculate the three-point formula Jackson subcutaneous fat was used.
Fat percent = (0.41563) × (all three parts) – ((square three parts) ×0.00112) + (0.03661× (age)) + 4.03653

### Research methodology

Bruce test as an exhaustive aerobic exercise is selected for the purpose of this study. After the description of research objectives and its method (the process of performing Bruce test, blood sampling, and the drug and placebo consumption), consent forms and exercise background, disease and drug abuse questionnaires submitted to the participants. Following the receiving of consent forms and questionnaires, some physical and physiological parameters of subjects including their age, height, weight, resting and maximum heart rate, body temperature, systolic and diastolic blood pressure, body fat percentage and body mass were measured and recorded. Subjects have performed Bruce exercise test protocol randomly in two consecutive weeks (1. First week: Placebo: n=12 and Cocoa: n= 8; 2. Second week: Placebo: n=8 and Cocoa: n=12). Accordingly, each of the athlete performed Bruce test twice (1.Following placebo consumption, 2.Following cocoa consumption). Immediately before, immediately and one hour following the performance of Bruce test, the second, third and fourth blood sampling was done. Then, to determine the amount of Plt, MPV and PDW changes, blood samples were assessed by Mindray Unit- cell Counter.

### Statistical Analysis

After the confirmation of homogeneity and lack of differences between initial data and the studied population (Shapiro Wilk test), a two- factor analysis of variance (ANOVA) for repeated measures with control group was used to evaluate the studied parameters changes during four time periods and groups mutual effect ( placebo and cocoa) and blood sampling procedures. In cases of the existence of differences between four time periods, Bonferoni test and to compare the difference between groups at each stage, t test was used. All the statistical analysis was done by SPSS16 Software at $p<0.01$.

**Table 1: Mean and standard deviation of participants' individual Characteristics (N = 20)**

| Measured indices | Mean ± SD |
|---|---|
| Age (years) | 22 ±1.3 |
| Height (m) | 178 ±2.6 |
| Weight (Kg) | 72 ±2.7 |
| Body mass index (Kg/m2) | 22 ±1.1 |
| Body fat percentage (%) | 22.5 ±1.2 |
| Resting HR (beat.min -1) | 70 ± 3.3 |
| Maximum heart rate (beats . min -1) | 187 ± 6.5 |
| Systolic blood pressure (mm Hg) | 12.5 ±0.7 |
| Diastolic blood pressure (mm Hg) | 8.3 ±0.5 |
| Temperature (° C) | 35.7 ±0.4 |
| Sports history (years) | 6.6 ±0.8 |
| Maximum oxygen consumption (ml/kg -min -1) | 53.7 ±1.5 |

## Results

Results suggested that there was a significant difference during cocoa consumption between first and second stages of studied parameters, which indicated a significant changes (significant decrease) of Plt, MPV and PDW following cocoa consumption ($p<0.01$). In addition, by cocoa consumption, a significant difference was found in relation to the amount of Plt, MPV and PDW changes immediately and one hour after the performance of Bruce training protocol between two complementary groups($p<0.01$). Therefore, cocoa influences on blood platelet parameters (Plt, MPV and PDW) in male soccer players. Finally a significant difference was observed between second and third stages of indexes in both complementary groups ($p<0.01$), that indicates the increase of Plt, MPV and PDW values following one session exhaustive aerobic exercise. So, one session of exhaustive aerobic exercise affect on the values of Plt, MPV and PDW in male athletes (tables 2, 3, 4, 5, 6, 7).

**Table 2: ANOVA tests with the control group (placebo and cocoa) in Plt index**

| Index | Sum of squares | Mean square | F | P |
|---|---|---|---|---|
| Plt | 5.15E + 011 | 3.74E + 011 | 1806.77 | E -045 2012 |
| Group*Plt | 2.72E + 011 | 1.98E + 011 | 955.72 | E -038 2028 |
| Error (Plt) | 1.08E + 011 | 207039864 | | |

**Table 3: Mean values between groups (placebo and Cocoa) in Plt index**

| index | differences between the two groups | standard error of the mean deviation | P |
|---|---|---|---|
| Immediately prior | 72000 | 1071 | 4E -041 |
| Immediately | 159815 | 5883.5 | 1.69E -026 |
| 1 hour later | 216000 | 2500 | 3.14 E -045 |

**Table 4: ANOVA tests with the control group (placebo and cocoa) in MPV Index**

| Index | Sum of squares | Mean square | F | P |
|---|---|---|---|---|
| MPV | 1173.9 | 469.5 | 1031.8 | 3.9 E -69 |
| Group* MPV | 384.9 | 153.95 | 338.4 | E -047 1.38 |
| Error (MPV) | 43.23 | 0.45 | | |

**Table 5: Mean values between groups (placebo and Cocoa) in MPV index**

| index | differences between the two groups | standard error of the mean deviation | P |
|---|---|---|---|
| Immediately prior | 1.41 | 0.15 | 7.8E -011 |
| Immediately | 5.6 | 0.24 | 9.7E -024 |
| 1 hour later | 7.7 | 0.26 | 2E -027 |

**Table 6: ANOVA tests with the control group (placebo and cocoa) PDW Index**

| Index | Sum of squares | Mean square | F | P |
|---|---|---|---|---|
| PDW | 13588.5 | 5477.5 | 863 | E -045 2.12 |
| Group* PDW | 3742.5 | 1508.6 | 237.7 | E -038 2.28 |
| Error (PDW) | 598.83 | 6.3 | | |

**Table 7: Mean values between groups (placebo and Cocoa) in PDW index**

| Index | differences between the two groups | standard error of the mean deviation | P |
|---|---|---|---|
| Immediately prior | 5.5 | 0.63 | 1.8E -010 |
| Immediately | 16.25 | 0.63 | 1.2E -025 |
| 1 hour later | 25 | 0.91 | 1.2E -026 |

## Discussion and Conclusion

### A. Incremental exhaustive exercise and Plt, MPV and PDW parameters

The data analysis of this study has indicated that one session of an incremental exhaustive and intense exercise ( Bruce test) caused a significant increase of blood Plt, MPV and PDW rates in athlete male subjects ($p<0.01$). The increase of platelet and therefore their activation due to intense physical exercises will raise the risk of heart attacks (Wang et al, 2005). Although according to the present evidence, it has been shown that physical exercise can alter the number and function of platelets, the variety of applied exercise methods and protocols in researches have made it difficult to investigate the extent and importance of these changes (Aldemir et al., 2005). However, it is assumed that physical exercises in various intensities affect on platelet function differently. In addition, the present study has investigated not only sedentary, athlete and prepared healthy subjects but also the patients including cardiac patients (Aldemir et al., 2005). Many researchers have stated that even though different experimental methods and training protocols led to similar results, but it is obvious that intense physical activity affect on the platelet function including its activation, aggregation and adhesion. However, it is well established that following exercises blood fibrinolysis system will increase in order to make balance between exercise possible effects on blood coagulation and platelets function changes (Aldemir et al., 2005). However, Plt increase may be due to the new platelets release from spleen vascular substrates, bone marrow or pulmonary circulation vascular vessels and lungs (Lars, 2005), or it may be due to the increase of body temperature, sweating, alpha receptors concentration upon platelets membranes and also the increase of plasma catecholamines concentration (Hurlen et al., 2000). Otto et al (1985), shown that the injection of adrenalin causes a strong contraction of spleen, in which more than one third of platelets is stored), and this (adrenalin increase) is the main cause of high blood Plt raise during physical exercises. On the other hand, Peters (1997) declared that the crushing of trapped megakaryocytes in bone marrow and lungs or their removal from liver and lungs are influential in exercise induced thrombocytosis (blood Plt increase). So it can be stated that the type, sex, and age of participants, their number and readiness, the type and time periods of training protocol and exhaustive exercise and blood sampling time are among the possible influential factors in different researches results. Intensity of exercises is an important factor which influences on different functions of platelets. While moderate physical exercise has no effect on platelets, intense exercises highly influence the platelets in healthy or even patient subjects (Aldemir et al., 2005).Exercise induced increase of MPV may be caused through the destruction of smaller platelets at initial hematopoietic levels due to the topical forces in the vessel wall and protection of blood greater platelets or the direct severe release of blood greater platelets (Yilmaz et al., 2004). Tong et al (1987) stated that by platelets formation stimulation through physical exercise and other

factors, MPV will be increased. The mean platelets volume compared with the normal platelets cycle, is the most accurate tool for measuring the platelets' size. The measurement of this index reflects the level of platelets stimulation and the amount of their production, and therefore, it is considered as a simple marker for activation and their activation rate. The increase of MPV rate during platelet severe activation as happened in cardiac patients, may be resulted from a change in fragmentation patterns of megakaryocytic cytoplasm, and this reflects the platelets biological changes in order to maintain homeostasis against the degradation and removal of platelets (Yilmaz et al., 2004) .In studies by (Karpatkin et al., 1969; Corash et al., 1977) it has been demonstrated that greater platelets which have been specified by increased MPV are more active than smaller platelets in regards to metabolic and enzymatic power and have greater and faster aggregation. PDW increase can be resulted from the associated mechanisms of vascular wall or the base behavior and function of platelets during the hematopoiesis process (Yilmaz et al., 2004). However, the exact mechanisms responsible for the increase of MPV and PDW through physical exercises are not specified. But it may be related to the release of big and young platelets especially from spleen to the blood (Ahmadizad et al., 2003).

**B. Cocoa and Plt, MPV and PDW indexes**

Data analysis of the research indicated that cocoa consumption influence on the blood Plt, MPV and PDW parameters in male athletes and results into their significant decrease ($p<0.01$). As a confirmation to the decrease of Plt and MPV following the cocoa consumption, (Murphy et al, 2003), despite the effect of the type, sex and the number of participants (13 healthy female with mean age of 40±9 years) and also the protocol and the amount of cocoa consumption (234 mg, for 28 days) with the present study, have shown the decrease of Plt and MPV following the cocoa consumption. However, this researcher in a study on 15 healthy male (mean age of 47±4 years) with the cocoa consumption (6 mg cocoa for 28 days), has also reported the increase of Plt and MPV. The difference of the obtained results may be due to the different factors such as the type, sex, age and the number of participants and also the protocol and the amount of cocoa consumption. So that, in the present study 20 young male athletes with the mean age of 23 years participated. Meanwhile the dosage of cocoa consumption was 19 g and the subjects consume this amount for only one meal. Cocoa and its components including flavonoids may result into the decrease of Plt through the influence on new platelets release from vascular beds of the spleen, bone marrow and or pulmonary circulation Vessels within the vascular and lungs, or may be through the reduction of Alpha receptors concentration on platelets membranes (Lars, 2005; Sies et al., 2005; Stoclet et al., 2004). In addition, the MPV value as a determining factor of platelets reactivity reflects the level of platelets formation and stimulation, so it can be said that cocoa reduces the level of Plt formation and stimulation through the reduction of MPV value (Stoclet et al., 2004).
There is no report about the influence of cocoa on PDW values. The decrease of MPV and PDW due to cocoa consumption can be resulted by the associated mechanisms of different properties and components of cocoa with vascular walls or it may be related to the influence of cocoa on the base behavior and function of platelets during the hematopoiesis process (Sies et al., 2005; Murphy et al., 2003). Rein et al (2004) stated that the mechanism of platelets function decrease through cocoa consumption is due to the elimination of platelets sensitivity to agonists. Another proposed mechanism for the reduction of platelet function is the LDL oxidation decrease through the cocoa consumption and therefore the decrease of Citric acid. The increase of Citric acid levels in blood results in the disorder of blood anti-oxidant capacity (Sies et al., 2005). Because of the existence of polyphenols (nutrients with anti-oxidative properties) in cocoa and therefore its anti- oxidant features, it decreases the bone marrow activation as a reaction to maintain the blood homeostasis, and reduces the blood platelets through sending of some fewer Megakaryocytes (Stoclet et al., 2004; Singh et al., 2006). Most of the physiological effects of cocoa resulted from the prevention or inhibition of cellular oxidation and antioxidant system improvement and therefore the elimination of reactive oxygen species (Sies et al., 2005). However, there is not so much information about the attraction process, different mechanism and functions of cocoa and its flavonoidsin human body, and the exact mechanisms of cocoa and its flavonoids for the inhibition of platelets activation is not clearly specified.

**C. Cocoa, incremental exhaustive exercise and Plt, MPV, PDW indexes:**

Cocoa consumption also influenced on the values of blood Plt, MPV and PDW in male Athletes and caused a significant decrease of them immediately and one hour following one session

incremental exhaustive exercise ($p<0.01$). The only study done in this regard Singh et al., (2006) should be investigated from two perspectives. First, by the study on athlete and sedentary subjects, this researcher concluded that cocoa consumption cannot reduce the level of Plt following physical exercises. In other words, cocoa was not able to inhibit the increase of Plt factors after physical exercises. Second, cocoa consumption in athlete subjects caused the reduction of MPV values. While the consumption of the same amount of cocoa in sedentary subjects couldn't prevent the increase of MPV level. The difference of obtained results may be related to factors such as type and number of subjects, protocol and the amount of cocoa consumption and also type, intensity and duration of physical exercise. Because physical exercises with diverse intensities affect the platelets function in a different manner. In the study by Singh et al, (2006) eight ready and eight sedentary cyclists had participated, who consumed daily 240mg cocoa in one week period (168g in the entire research period). While the participants of the present study consisted of 20 soccer players who consume 18.75 g cocoa in one meal. Mean while the training protocol in Singh et al, (2006) study included one hour sub-maximal cycling by the intensity of 70% VO2max, and in fasting state. In contrast, training protocol in the present study consisted of Bruce test with lower time and higher intensity and in non-fasting state. The level of participants' physical fitness is also influential in the observed results differences. However, the reduction of platelet indexes (Plt, MPV and PDW) values due to cocoa consumption following the exercises in this research, may be related to the decrease of new platelets release from vascular beds and or pulmonary circulation Vessels within the vascular and or reduction of body temperature, the reduction of alpha receptors concentration on platelets membranes and the reduction of plasma catecholamine concentrations (Stoclet et al., 2004). Another possible mechanism for the reduction of platelet variables through cocoa consumption following maximal exercises can be the increase of blood PH due to Lactate decrease (Singh et al., 2006). Heavy physical exercises will increase the production of thrombin from vascular walls, which performs as a factor for the escalation of platelet activation and function. It can be said that platelet decrease in this study may be caused by the influence of cocoa on vascular walls and the reduction of thrombin production (Sies et al., 2005; Stoclet et al., 2004). However, for the investigation of cocoa consumption effect alone or in combination with physical exercises on blood parameters examined in this study, further researches will be necessary (given the factors such as type, sex and age of participants, number and their level of readiness, time period and the type of training protocol and exhaustive exercise, intensity, duration and frequency of exercises, the amount of consumed cocoa and its consumption protocol) in the future to discuss and conclude with more certainty about the influential factors on the decrease or increase of blood Plt, MPV and PDW.

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