Spor Hekimliği Dergisi
Cilt: 45, S. 45-56, 2010

SUBMAXIMAL EXERCISE, BLOOD PRESSURE, HEART RATE, AND HAEMATOLOGICAL RESPONSES TO SHORT PERIOD DARK CHOCOLATE INTAKE IN SWIMMERS

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SUMMARY

The aim of this study was to assess the influence of antioxidant rich dark chocolate supplementation on blood pressure (BP), heart rate (HR), red blood cells (RBC), haemoglobin (Hb), and haematocrit (Hct) in response to submaximal exercise in male swimmers. Eleven competitive swimmers aged 18-21 years participated in a randomised experimental protocol consisting of a 10 days washout period with no chocolate intake, and a 10 days supplementation period with daily 50 g dark chocolate ingestion. At the end of each period, two submaximal bicycle ergometer tests (Test I and Test II) of 15 min each (10 min at 60% VO_2max, and 5 min at 90% VO_2max) were conducted to induce oxidative stress. HR was monitored at baseline, during the tests and following 3 min of recovery. Blood samples were obtained before and following the submaximal tests, and BP was measured at rest and at the end of each period. Significantly lower ($p<0.005$) systolic and diastolic BP levels were found after 10 days of chocolate intake. HR during Test II was lower and displayed significantly faster decline in the first 3 min of recovery. RBC, Hb and Hct were negligibly lower after performing Test I; whereas slight, but significant increases were observed in these parameters in response to Test II. In conclusion, short-term dark chocolate supplementation may decrease BP and HR levels. It may also modulate beneficial changes in RBC, Hb and Hct in response to submaximal exercise-induced oxidative stress.

Key words: Dark chocolate, oxidative stress, blood pressure, heart rate, red blood cells, haemoglobin, exercise, swimming

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ÖZET

YÜZÜCÜLERDE KISA SÜRELİ ESMER ÇİKOLATA KULLANIMININ SUBMAKSİMAL EGZERSİZ, KAN BASINCI, KALP ATIM HIZI VE HEMATOLOJİK PARAMETRELERE ETKİLERİ

Bu çalışmanın amacı, antioksidan özellikleri yüksek olan esmer çikolata desteğinin erkek yüzücülerde submaksimal egzersiz testinde kan basıncı (BP), kalp atım hızı (HR), alyuvar (RBC), hemoglobin (Hb) ve hematokrit (Hct) parametrelerine etkilerini değerlendirmekti. Yaşları 18-21 arası değişen 11 üst düzey müsabık rastgele şekilde bir deney protokolüne katıldılar. Protokol 10 günlük çikolata alımı içermeyen ve aynı sürede günlük 10 g esmer çikolata alımı içeren periyotlardan oluşuyordu. Her bir periyodun sonunda oksidan stres yaratmak için 15 dk süreli (%60 VO_2max'da 10 dk ve %90 VO_2max'da 5 dk) submaksimal bisiklet ergometresi testi (Test I ve Test II) uygulandı. HR dinlenmede, testler süresince ve toparlanmanın ilk 3 dk'sı boyunca monitorize edildi. Testlerin öncesi ve sonrasında kan örnekleri alındı. BP düzeyleri de dinlenmede ve her iki periyodun sonunda ölçüldü. Esmer çikolata alımı sonrasında anlamlı ölçüde düşük ($p<0.005$) sistolik ve diastolik BP düzeyleri saptandı. Test II sonrası HR daha düşüktü ve toparlanmanın ilk 3 dk'sında anlamlı düşüş ortaya koydu. RBC, Hb ve Hct düzeyleri Test I sonrasında belirsiz ölçüde düşükken, Test II sonrasında bu parametrelerde hafif ama anlamlı artışlar gözlendi. Sonuç olarak, kısa süreli esmer çikolata desteğinin kan basıncı ve kalp atım hızı değerlerini düşürebileceği; ayrıca submaksimal egzersizin tetiklediği oksidan strese RBC, Hb ve Hct düzeylerinde olumlu yanıtların verilmesine aracı olabileceği söylenebilir.

Anahtar sözcükler: *Esmer çikolata, oksidan stres, kan basıncı; kalp atım hızı, alyuvar, hemoglobin, egzersiz, yüzme*

INTRODUCTION

Intensive physical exercise can greatly increase the production of reactive oxygen species (ROS) and cause oxidative stress. Oxidative stress is characterized by an imbalance between ROS generation and cell antioxidant defense system activity. This system includes endogenous compounds synthesized by the body (uric acid, glutathione, bilirubin, catalase, superoxide dismutase, glutathione peroxidase, etc.), and exogenous compounds consumed with food (carotenoids, tocopherols, bioflavonoids, etc.). These compounds are able to deactivate ROS (30,32). Effectiveness of the antioxidant system depends on the consumed food, endogenous production of enzyme- and non-enzyme antioxidants, and can be changed by exercise, training program, age and diet (8).

Oxidative stress induced by intensive exercise may damage cellular components such as lipids, proteins, heat shock proteins and DNA, and therefore reduce the working capacity (4). It may also have harmful effects on cell membranes of skeletal muscle, heart, liver and erythrocytes. Erythrocytes, due to their high content of fatty acids, rich supply of O_2, and the presence of transition metals Cu^{2+} and Fe^{2+}, are particularly prone to oxidation. ROS can attack their membranes, causing oxidation of lipids and proteins, leading to haemolysis (33).

Different antioxidants supplementation strategies aim at reducing oxidative stress caused by physical activity, and its harmful effects such as reduced work capacity, inflammatory processes that lead to fatigue, prolonged recovery and overtraining (10,19,23,27,32). Studies with dietary interventions and supplementation with chocolate in particular, attracted attention due to dark chocolate's high content of flavanols (catechin and epicatechin) and their procyanidin oligomers. Flavonoids may cause various biological effects on circulatory and immune systems. They may reduce the risk of cardiovascular disease. Cocoa flavanols acting as antioxidants may be involved in this protection through various mechanisms such as reducing blood pressure by increasing nitric oxide production and improving the function of vascular endothelium, decreasing platelet reactivity, and inhibiting inflammatory processes (2,5).

The aim of the study was to assess the effect of short-term intake of dark chocolate on arterial blood pressure (BP), heart rate (HR), red blood cells (RBC), haemoglobin (Hb) and haematocrit (Hct) in swimmers undergoing submaximal exercise test.

MATERIAL and METHODS

Subjects: Highly trained competitive swimmers (n=11) from the National Sport Academy Sofia team were recruited for this study. Their mean age was 19.1 ± 1.0 years. All participants volunteered and written consent was obtained prior to the study. The swimmers were interviewed about their current health status and medication by a health professional. Experimental protocols were approved by the Research Board of the National Sports Academy Sofia and were conducted in accordance with the Helsinki Declaration for Ethical Treatment of Human Subjects.

Experimental design: At the beginning of the study, resting baseline measurements of BP, HR and anthropometric indexes were taken. Participants agreed to adhere to their usual diet throughout the study, and refrain from consumption of cocoa products other than those provided for supplementation.

Aerobic capacity (VO_2max) and maximum work rate (Wmax) of each subject were determined on a bicycle ergometer through a graded protocol (14) with initial work load of 60 W at 60 rpm, and increases of 30 W every 1.5 min until either exhaustion or the subjects' oxygen uptake reaching a plateau (14). Gas analysis was done using an Oxycon analyzer (Erich Jaeger GmBH & Co, Wuerzburg, Germany).

As swimmers were their own controls, a randomised experimental protocol including two 10 days periods was used: a washout period with no chocolate intake, and a supplementation period with daily 50 g ingestion of dark chocolate. During the supplementation period, chocolate was ingested under supervision at 11:00 am, following the morning training session. The product used was original rich dark chocolate (New Nestlé Club, 60% cocoa) containing sugar, cocoa mass, cocoa butter, butter, emulsifier (soya lecithin), and traces of milk, peanuts, eggs and gluten. Manufacturer's nutritional data per 100 g was as follows: energy 2133 kJ, protein 5.2 g, fat 31.3 g, carbohydrate 51.9 g and magnesium 121 g.

In order to cause oxidative stress, each participant took part in two trials of 15 min submaximal exercise tests on a bicycle ergometer at an intensity of 60% Wmax in the first 10 min, increasing to 90% Wmax in the last 5 min. The first test (Test I) was performed at the end of the washout period and the second one (Test II) upon completing the chocolate supplementation period. HR, BP and body mass index (BMI) were measured at baseline and at the end of both periods. HR was recorded during the two exercise tests and during the first 3 min of recovery. Blood samples were collected before and immediately following submaximal tests for the determination of RBC, Hb and Hct. Analysis was performed using analyzer (Cell-Dyn 3500, Abbott) at the Cibalab accredited laboratory, Sofia, in accordance with EU standards of good laboratory practice.

During both supplementation periods, training loads and diets were registered. For food consumption evaluation, a 7-day diet questionnaire prepared according to Karvetti and Knuts (15) and Pao and Cypel (22) was used, and was distributed with precise and clear instructions on how to register consumed food and drinks. Data were converted into consumed nutrients using food composition tables (7,17,24). Basal metabolic rate (BMR) and metabolic rate (MR) were calculated according to Thompson and Manore (29), average daily energy need was calculated applying the Cunningham equation, used by the same authors.

Statistical analysis: Results are presented as means (X) ± SEM, since repeated measurements of the parameters were conducted in line with similar supplementation and diet intervention studies (9,12,28,32,33). Statistical significance of differences was calculated using paired Students' test for dependent samples. Significance was considered at $p<0.05$.

RESULTS

Physical characteristics of the swimmers are given in Table 1. Body fat (BF) and active body mass (ABM) were calculated according to Durnin and Womersley (6). The group was largely homogeneous in terms of physical development. No significant changes in body weight, BMI and other physical characteristics were registered during the entire study.

Table 1. Physical characteristics of the swimmers (X ± SEM)

Characteristics	X ± SEM
Height (cm)	181.5 ± 1.6
Body weight (kg)	73.9 ± 1.0
Basal metabolic rate (BMR) (kcal. d^{-1})	1650 ± 76
Metabolic rate (MR) (kcal. d^{-1})	2912 ± 249
Calculated energy expense (kJ.d^{-1})	13156 ± 311
VO_2max (ml. min^{-1}.kg^{-1})	62.5 ± 1.9
Body mass index (BMI) (kg.m^{-2})	22.4 ± 0.2
Body fat (BF) (%)*	12.6 ± 0.5
Body fat (BF) (kg)*	9.3 ± 0.4
Active body mass (ABM) (kg)*	64.6 ± 1.2
Left hand force (kg)	51.1 ± 2.1
Right hand force (kg)	53.2 ± 1.8
Waist circumference (cm)	77.4 ± 0.5
Wrist circumference (cm)	16.9 ± 0.2
Arm circumference (cm)	29.0 ± 0.2
Bust at pause (cm)	92.0 ± 0.6
Bust at maximum inhalation (cm)	98.0 ± 0.6
Bust at maximum exhalation (cm)	86.0 ± 0.4

Analysis of individual records of completed training programmes showed that during the experiment, the swimmers had performed moderate to high volume training loads, averaging 6.12 km per day with an intensity depending upon the design of training.

Dietary intake of the athletes during the two study periods are presented in Table 2. There were no statistically significant differences in the diet during both periods. Data on BMR, MR and estimated energy expense (EE) are given in Table 3. Upon comparing baseline, end of the washout and supplementation periods, a significant increase of BMR and MR were found following the supplementation period, without any appreciable change in the estimated energy needs.

Table 2. Dietary consumption of the swimmers (X ± SEM)

Periods	Washout	Supplementation
Calculated energy (MJ/day)	14.0 ± 0.9	14.1 ± 0.7
Proteins (g)	147 ± 9	145 ± 8
Carbohydrates (g)	417 ± 14	429 ± 11
Fats (g)	112 ± 8	116 ± 7
Fibers (g)	38.0 ± 2.1	36.0 ± 2.4

Table 3. Basal metabolic rate (BMR), metabolic rate (MR) and calculated energy expense (EE) at rest and during the investigation (X ± SEM)

Measurements	BMR, kcal/day	MR, kcal/day	EE, kJ/day
Baseline	1650 ± 76	2912 ± 249	13156 ± 311
Post-washout	1602 ± 44	2678 ± 88	12766 ± 105
Post-supplementation	1970 ± 70*,**	3047 ± 30*,**	12753 ± 113

*p<0.05 wrt baseline; **p<0.001 wrt washout period

Changes in HR during the tests are presented in Figure 1. Results revealed that the tests are of submaximal nature during the first 10 min, when the load is at 60% of Wmax, and the effort was close to maximal working capacity during the last 5 min. HR was over 180 bpm at the end of both tests in most cases, over 190 bpm for two swimmers in Test I, and reached 204 bpm in one respondent at the end of Test II. Average HR tended to be lower during Test II, and significantly faster recovery during the first 3 min was observed after Test II. The case of a swimmer featuring very good working capacity, who worked with lower HR especially in the submaximal stage of Test II is displayed in Figure 2.

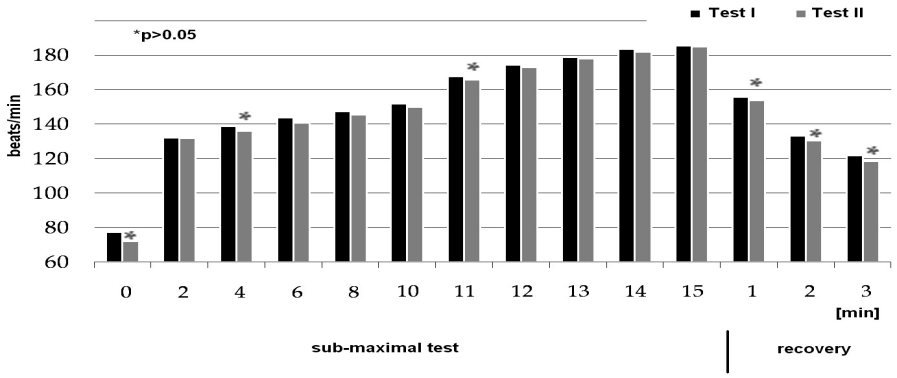
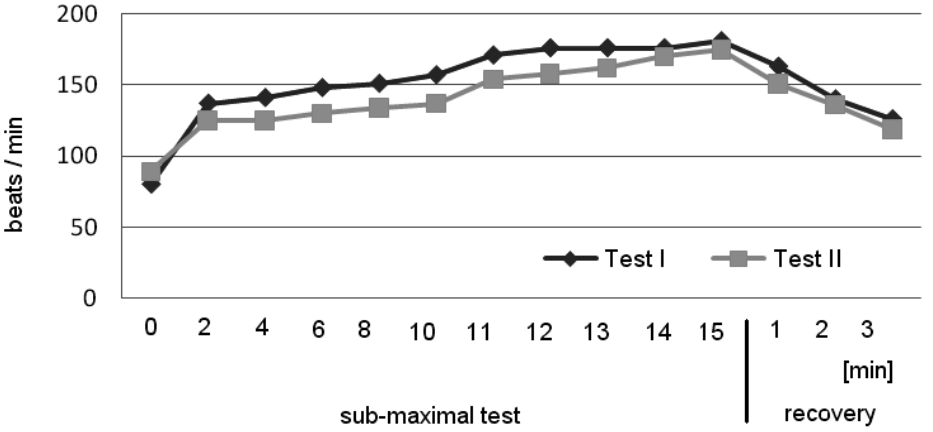


Fig. 1. Average values of heart rate during submaximal Test I and Test II



Test I: End of washout period; Test II: End of supplementation period

Fig. 2. Heart rate of a swimmer (GK) during Test I and Test II

The effects of dark chocolate treatment on some indices of the CV system, including pulse pressure (PP) and systolic tension time (TTsys) measured at rest are presented in Table 4. At the end of the experiment, average levels of systolic (BPsys) and diastolic (Bpdia) blood pressure of the swimmers were reduced significantly ($p<0.05$) by 8.9 mmHg and by 12.5 mmHg respectively, compared with baseline levels. When resting BPsys and BPdia before Test I and Test II were compared, significant decreases of 7.9 mmHg and 10.3 mm Hg respectively, were observed. Systolic tension time was also significantly reduced.

Table 4. Cardiovascular system indices at rest and at different stages of the experiment (X ± SEM)

Period	HR (bpm)	BPsys (mmHg)	BPdia (mmHg)	PP (mmHg)	TTsys (mmHg.bpm)
Baseline	62.2 ± 1.9	125.8 ± 4.0	75.5 ± 3.5	50.0 ± 1.4	7822 ± 342
Washout	62.8 ± 1.0	126.0 ± 2.4	72.3 ± 1.8	50.6 ± 1.0	7912 ± 153
Test I	62.7 ± 0.5	124.8 ± 1.2	73.3 ± 1.0	47.6 ± 1.0	7738 ± 107
Test II	61.7 ± 0.4	116.9 ± 2.1*,**	63.0 ± 1.9*,**	53.9 ± 1.9*,**	7228 ± 142*,**

*$p<0.05$ wrt baseline and washout periods; **$p<0.05$ wrt Test I

Changes in RBC count, Hb, Hct and blood oxygen capacity (BOC) are summarized in Table 5. Following Test I, RBC, Hb and Hct revealed a slight and insignificant decrease, whereas after completing Test II, the levels of these parameters increased ($p<0.05$). These changes were within the reference value ranges. No changes were observed in BOC.

Table 5. Red blood cells, haemoglobin, haematocrit and blood oxygen capacity responses to submaximal Tests I and II (X ± SEM)

Period	Sampling	RBC (10^{12}/L)	Hb (g/L)	Hct (%)	BOC (mlO_2/100ml)
Washout, Test I	Pre-test	5.29 ± 0.08	159.0 ± 2.7	46.7 ± 0.9	21.30 ± 0.04
	Post-test	5.17 ± 0.06	157.4 ± 1.6	45.0 ± 0.3	21.48 ± 0.27
Suppl., Test II	Pre-test	5.35 ± 0.06	159.4 ± 1.6	47.0 ± 1.0	21.32 ± 0.24
	Post-test	5.30 ± 0.05*	160.3 ± 2.0*	45.5 ± 0.6*	21.14 ± 0.26

*p<0.05 comparison after exercise between Test I and Test II values

DISCUSSION

The high content of antioxidants in chocolate may counteract oxidative stress caused by physical exercise and its damaging effects on metabolic processes. This statement is consistent with studies of several authors who have found that consumption of flavonoid-rich dark chocolate reduces oxidative stress, decreases blood pressure and increases serum antioxidant capacity, lowers the level of lipid peroxidation, reduces oxidation of LDL and increases HDL-cholesterol (9,13,16,20,25,28).

In this study, heart rates recorded during the submaximal tests proved that the intensity of the exercise is high enough to cause oxidative stress (30). After consuming chocolate for a short term, significant heart rate improvements in swimmers during the second exercise test and the recovery period may be considered as a response indicative of amended efficiency of the cardiovascular system. In addition, metabolic rates were increased. These positive effects are likely to be related to the action of flavanols in the dark chocolate.

The lower blood pressure level results of the study are in line with findings of other authors who have observed a similar effect of chocolate consumption in young soccer players (9), in normotensive individuals (1,12), and those with moderate hypertension (11,28). It should be noted that some researchers (3,26) have found no changes in mean arterial pressure after administration of chocolate. It is known that high blood pressure is a risk factor for many cardiovascular diseases. Its decline even in levels close to or within the normal range is beneficial for health and sports performance (9).

The findings indicating slight increases within the reference range of RBC and haemoglobin levels following the dark chocolate intake period should be noted. Experimental data about the influence of dark

chocolate on these parameters is scarce, but some effects of cocoa flavonoids (33) and exercise (21) have been reported. There is evidence that some proteins (haem-containing, immunoglobulin and albumin) may undergo oxidative damage established by appearance of attached carbonyl groups, when exposed to oxidative stress. Due to their limited biosynthetic capabilities and poorly developed repair mechanisms, erythrocytes can accumulate changes at the molecular level leading to damages in their cell membrane components (18), and haemolysis (33).

Enhanced resistance to haemolysis has been related to modulations in eicosanoid metabolism and lower leucotriene to prostaglandin ratio (31,33). In RBC of trained long-distance skiers, low levels of the enzyme superoxide dismutase (SOD) after implementation of a graded exercise test to exhaustion have been found (31). On the other hand, no changes in SOD activity in erythrocytes have been observed in untrained individuals following an 8 weeks moderate intensity cycling programme, and in trained athletes after moderate exercise (18). The combined effects of dark chocolate supplementation and strenuous exercise on RBC and Hb need to be further investigated.

According to Kelishadi (16), the rough estimates for the amount of flavanol-rich chocolate, which should be consumed to achieve acute or chronic effects, are respectively 38 g and 125 g. After consumption of dark chocolate, epicatechin is rapidly absorbed. Depending on the quantity of chocolate consumed, its plasma concentration reaches levels in the range of 0.3-0.7 μmol/L, similar to that observed after consumption of apples, onions and tea. It can reach 1.0 μmol/L in less than two hours, but returns to baseline within eight hours (25,31).

The positive influence of chocolate's epicatechin, cathechin and procyanidins on metabolism (11,12), metabolic rate (16), vessel dilation (2,28), membrane protection (31,33), inflammation and immunity (13) are mediated via modulating mechanisms in various metabolic pathways. Based on the above mentioned data and our experimental results revealing improved metabolic rate, lower blood pressure and heart rate, it is assumed that the daily intake of 50 g dark chocolate by athletes is a quantity sufficient to cause the observed beneficial effects.

In conclusion, short-term supplementation with dark chocolate was found to possess hypotensive effects, improves the functioning of the cardiovascular system, and may modulate favorable changes in erythrocytes and haemoglobin in response to oxidative stress caused by submaximal exercise.

Acknowledgements: This study is part of a joint research project between the National Sport Academy, Sofia, Bulgaria and the University of Zululand, South Africa, and was funded by the National Sport Academy, Sofia. We thank Prof. L. Dimitrov (Rector) and Prof. P. Bonov (Vice-Rector) of the National Sport Academy, Sofia for their support, as well as Mrs. Y. Vladimirova, Ms. L. Ocholla and Mrs. K. Enslin for their help and assistance.

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