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INFLUENCE OF DARK CHOCOLATE ADMINISTRATION ON URIC ACID, LIVER ENZYMES, LACTATE AND GLUCOSE CHANGES INDUCED BY SUBMAXIMAL EXERCISE IN ATHLETES

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ABSTRACT

Objective. *To assess the influence of dark chocolate administration on uric acid, liver enzymes, glucose and lactate responses to summaximal exercise test in male swimmers.*
Subjects. *Eleven competitive swimmers from the athletic club Academic, Sofia volunteered for the study.*
Design. *A randomised study of two periods of 10 days (washout period without chocolate and administration period of 50 grams dark chocolate daily) was carried out. Each subject took part in two trials of 15 min submaximal exercise test on a bicycle ergometer consisting of two bouts of 10 min at intensity 60% VO_2max and 5 min at 90%VO_2max conducted without an interval to induce oxidative stress.*
Results. *Uric acid after performing pre-administration period submaximal exercise was elevated by 11.7% ($p<0.05$) but dropped by 10.9% in response to submaximal test after 10 days of chocolate intake. Blood lactate increased immediately following the submaximal tests up to 6.6 mmol.l^{-1} and 6.1 mmol.l^{-1} respectively. Lactate was significantly lower after the dark chocolate treatment. Following submaximal tests pre-administration period glucose was elevated to 5.65 mmol.l^{-1} whereas post chocolate administration glucose levels were reduced significantly. Liver enzymes changes were found within the upper reference range. Aspartate-aminotransferase levels were raised by 14.8% after both tests. No changes in γ-aminotransferase, triglycerides and cholesterol were found.*
Conclusion. *Dark chocolate short-term administration could modulate and benefit metabolic changes in uric acid and lactate in response to submaximal exercise-induced oxidative stress.*

Key words: Swimmers; Exercise; Oxidative stress; Uric acid; Glucose; Lactate.

INTRODUCTION

Exercise can produce an imbalance between reactive oxygen species (ROS) and the innate antioxidant defense system resulting in oxidative stress. Antioxidant defense system consists of glutathione, antioxidant vitamins and antioxidant enzymes (Li Li Jt, 1995: 18). ROS production (superoxide anions, hydrogen peroxide and hydroxyl radicals), detected by electron spin resonance, has been measured in the liver and muscle fibres in rats and human blood samples after submaximal exercise to exhaustion (Ashton *et al*., 1998: 77). Oxidative

stress does not always result in oxidative damage to the tissues but it may lead to potential damage to lipids, proteins, heat shock proteins and DNA, consequently decreasing athletic performance (Deaton & Martin, 2004: 2). Exhaustive exercise can cause some detrimental effects on the cell membrane in various organs (heart, skeletal muscle and liver). Liver damage can be assessed by the levels of plasma aminotransferases. Submaximal exhaustive exercise at 90% VO_2 max was found to result in elevated formation of uric acid in human skeletal muscle (Sevanian *et al.,* 1991:54, Hellsten *et al.,* 2001: 31). Warning *et al.* (2003) provided evidence that uric acid functions as an antioxidant by inhibiting the iron catalysed oxidation of ascorbic acid by binding iron and the scavenging ROS such as hypochlorous and hydroxyl radicals. Whitehead *et al.* (1992) estimated that uric acid elevation can account for nearly one-third of the plasma total antioxidant capacity (TAC) increase.

The positive outcomes of antioxidant supplementation on oxidative stress and performance in athletes have been reported (Jakeman & Maxwell, 1993: 67; Sachek & Blumberg, 2001: 17; Sachek *et al*., 2003: 34; Bonina *et al*., 2005: 25; Watson *et al*. 2005: 37). On the other hand, a number of studies have mentioned conflicting results (Sen, 2001: 33; Childs *et al*., 2001: 15; Urso & Clarkson, 2003: 189). Dark chocolate is rich in flavinoids, providing positive cardiovascular and metabolic effects by increasing serum total antioxidant capacity, HDL-cholesterol concentration and reducing LDL oxidation (Wan *et al*., 2001: 76). The effects of chocolate bar supplementation on plasma glucose, triglycerides, cholesterol, lactate, urea and free fatty acids have been investigated (Chen *et al.,* 1996: 9; Djarova *et al*., 2007: 1). The purpose of this study was to assess the influence of dark chocolate administration on uric acid, liver enzymes, glucose and lactate responses to submaximal exercise test in male swimmers.

METHODS

***Subjects*:** Eleven highly trained competitive swimmers aged 17-21 years participated in the study. All subjects were volunteers and a written consent form was obtained prior to the study. Experimental procedures were conducted in accordance with the Helsinki Declaration for Ethical Treatment of Human Subjects and approved by the Research Board of the National Sports Academy, Sofia. Each subject completed a maximal exercise test to ascertain maximal oxygen uptake (VO_2max). The VO_2max test was done on bicycle ergometer using a graded protocol (Iliev, 1986) at initial work load of 60 watts at 60 revolutions per minute and an increase by 30 watts every 1 min and 30 sec until exhaustion and/or the subjects oxygen uptake reached a plateau. The gas analysis was done using Oxycon, Yeger. After the initial VO_2 max test the subjects were advised to maintain their habitual diet and to refrain from ingesting chocolate and cocoa products other than that provided during the study.

***Research design*:** Baseline measurements were taken at the beginning of the study at rest for determination of of uric acid (UA), aspartate-amininotransferase (As-AT), alanine-aminotransferase (Al-AT) and γ-aminotransferase (γ-AT). Blood samples were collected from the antecubital vein into vacutainers and analysed in the accredited laboratory Cibalab, Sofia, Bulgaria according to the European Union standards of good laboratory practice using Hitachi 911, Japan. Glucose, triglycerides, cholesterol and lactate were determined in arterialised capillary blood using an Accutrend^R testing device and lactate meter manufactured by Roche International. A randomised study of two periods of 10 days (washout period without chocolate and administration period of 50 grams dark chocolate intake daily) was carried out. During the administraion period chocolate was ingested every day at 11:00 after the morning

swimming training session under supervision. The product used was New Nestle Club original rich dark chololate (70% cocoa) containing sugar, cocoa mass, cocoa butter, butter oil (soya lecithin) and traces of milk, peanuts, eggs, gluten. The nutritional information per 100 grams obtained from the manufacturer Nestle is as follows: energy 2133 KJ, protein 5.2 grams, fat 31.3 grams, carbohydrates 51.9 grams, magnesium 121 grams. Pilot experiments done before the commencement of this study have shown that there is no acute effect of a single intake of 50 grams Nestle dark chocolate 30 min after ingestion at rest and immediately after performing submaximal test on the investigated parameters.

Each subject took part in two trials of 15 min submaximal exercise test on a bicycle ergometer. The test consisted of two bouts of 10 min at intensity 60% VO_2max and 5 min at 90% VO_2max conducted without an interval between them to induce oxidative stress. The submaximal tests were performed at 08:30 at the beginning of the administration period (pre-administration period submaximal testing) and at the end of the chocolate administraion period (post-chocolate administration period testing). The participants were used as controls of themselves. Blood samples for measurements of all parameters were taken at the end of the washout period and immediately after performing pre-administration and post chocolate administration submaximal tests.

***Statistical analysis*:** A paired *t*-test was used to analyse the statistical difference. The results are presented as mean ± SEM. Statistical significance was accepted at $p<0.05$.

RESULTS

The physical characteristics of the subjects are shown in table 1. No changes in the body weight and BMI were recorded during the period of the study. No statistically significant differences were found between baseline and washout period measurements.

TABLE 1. **PHYSICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS (N = 11)**

Characteristics	Mean ± SEM
Age (years)	19.1 ± 1.0
Height (cm)	181.5 ± 1.6
Weight (kg)	73.9 ± 1.0
Estimated energy expenditure ($KJ.d^{-1}$)	13156 ± 310.5
Maximal oxygen uptake ($ml.kg^{-1}.min^{-1}$)	62.5 ± 1.9
Body mass index (BMI) $kg.m^{2\ -1}$	22.4 ± 0.2

Uric acid and liver enzymes responses are summarised in table 2. Uric acid after performing pre-administration period submaximal exercise was elevated by 11.7% ($p<0.05$) above the upper limit of the reference range (420 $mmol.l^{-1}$) but dropped by 10.9% in response to the submaximal test after 10 days of chocolate intake. Aspartate-aminotransferase levels were raised by 14.8% after both tests. Slight increase ($p<0.05$) in alanine-aminotransferase was

noted. All changes were within the reference range. No changes in γ-aminotransferase were found.

TABLE 2. **URIC ACID (MMOL.L^{-1}) AND LIVER ENZYMES (U/L) IN RESPONSE TO SUBMAXIMAL EXERCISE TEST AFTER DARK CHOCOLATE ADMINISTRATION (MEAN ± SEM)**

	Uric acid mmol.l^{-1}	As-At U/L	Al-AT U/L	γ–AT U/L
Baseline measurements	417.33 ±39.22	28.31 ±2.84	23.60 ±1.63	19.81 ±1.86
Washout period measurements	404.55 ±22.63	26.5 ±1.51	21.95 ±1.67	17.93 ±1.49
Pre-administration period submaximal testing	451.82* ±28.92	31.05* ±1.47	25.19* ±1.20	17.95 ±1.25
Post chocolate administration period submaximal testing	402.64** ±14.51	31.49* ±2.25	23.29 ±1.63	18.75 ±1.11

* $p < 0.05$ compared to washout period values

** $p < 0.05$ pre-administration period submaximal testing vs post chocolate administration period submaximal testing .

Lactate, glucose, triglycerides and cholesterol responses to submaximal tests are presented in table 3. Blood lactate increased immediately following the submaximal tests up to 6.6 mmol.l^{-1} and 6.1mmol/l^{-1} respectively. Lactate was significantly lower after dark chocolate treatment. Following the submaximal tests, glucose was elevated significantly to 5.65 mmol.l^{-1} borderline values and reduced by 13.8% after dark chocolate treatment period. Slight changes in triglycerides within the reference range were recorded. No statistically significant differences were found in cholesterol levels.

TABLE 3. **LACTATE, GLUCOSE, TRIGLYCERIDES AND CHOLESTEROL (MMOL.L^{-1}) IN RESPONSE TO SUBMAXIMAL EXERCISE TEST AFTER DARK CHOCOLATE ADMINISTRATION (MEAN ± SEM)**

	Lactate mmol.l^{-1}	Glucose mmol.l^{-1}	Triglycerides mmol.l^{-1}	Cholesterol mmol.l^{-1}
Baseline measurements	2.08 ±0.12	4.97 ±0.15	1.18 ±0.11	4.04 ±0.10
Washout period measurements	2.15 ±0.11	4.63 ±0.23	1.04 ±0.12	3.87 ±0.06
Pre-administration submaximal testing	6.61 ±0.29	5.65* ±0.21	1.28* ±0.08	3.92 ±0.06
Post chocolate administration period submaximal testing	6.11** ±0.51	4.87** ±0.15	1.17 ±0.06	4.07 ±0.07

* $p < 0.05$ compared to washout values

** $p < 0.05$ pre-administration period submaximal testing vs post chocolate administration period submaximal testing

DISCUSSION

The flavinols in the dark chocolate act by decreasing ROS production and detoxifying oxygen intermediates, thereby reducing oxidative stress at molecular level. The content of flavinols and procyanidins in dark chocolate is 170 mg per 100 grams and its oxygen radical absorbance capacity (ORAC) is 13.1 units. Dark chocolate has the highest flavinol content and its ORAC capacity is much stronger compared with other phytochemical-rich foods such as prunes, raisins, garlic, blueberries, cranberries, strawberries, spinach and broccoli (Steinberg *et al*., 2003: 103; Kelishadi, 2005: 1).

The observed increased uric acid plasma concentrations after submaximal exercise test are in agreement with data reported for marathon runners, humans after eccentric exercise, sledge dogs after endurance exercise, horses after submaximal exercise and healthy adults (Deaton & Martin, 2004: 2; Warning *et al*., 2003: 105). Uric acid is produced from the breakdown of purine compounds in the muscle (Hellsten *et al*., 2001: 31) and its elevation is proportional to the exercise intensity and the training level. It was reported that there is an exercise intensity threshold where the reamination of IMP cannot keep pace with the deamination of AMP resulting in uric acid accumulation (Deaton & Martin, 2004: 2) and the enzyme xanthine oxidase acts as a mini-electron transport protein. Uric acid acts as an antioxidant and the mobilisation of the tissue antioxidant stores into the plasma is a widely accepted phenomenon that helps to maintain the plasma antioxidant status when needed (Margonis *et al*., 2007: 43). On the other hand, during the oxygen reperfusion of the muscle tissue following intensive exercise, it was found that in the muscle xanthine oxidase continues to convert hypoxanthine to uric acid utilising oxygen as the electron acceptor leading to superoxide build-up (Heunks & Dekhuijzen, 2000: 55). Therefore, high plasma concentrations of uric acid (above the reference range) after the submaximal test performed prior the beginning of the chocolate administration found in our study could be interpreted as an indicator of mobilization and maintenance mechanisms but not as signs of a higher potential of the natural antioxidant systems and TAC. In the present study ROS and TAC were not measured and the contribution of uric acid to TAC was not studied. The results of TAC measuring have been controversial either detecting increases in the oxidative stress after exercise (Childs *et al*., 2001: 15, Wan *et al*., 2001: 76) or showing no elevation in TAC in response to 30 min of submaximal exercise (Urso & Clarkson, 2003: 189). It was also found that TAC and some individual blood antioxidant markers were not significantly reduced by the restriction-antioxidant diet intervention when compared with high-antioxidant diet (Watson *et al*., 2005: 37).

The lower accumulation of uric acid in response to submaximal test performed after 10 days of chocolate intake could be considered as a beneficial effect due to flavinols-facilitated adjustment of counteracting “buffering” mechanisms between the nonenzymatic (uric acid) and enzymatic (xanthine oxidase) components of the antioxidant system and the exercise-induced ROS production.

It appears that flavinols present in the dark chocolate may contribute to the balancing of the scavenging activity of natural antioxidants by reducing the uric acid accumulation. This mechanism in turn may play a considerable role in the recovery period in the skeletal muscle leading to attenuated accumulation of superoxide anions.

No sign of liver damage was established in response to oxidative stress-induced exercise by measuring the activity of aminotransferases. It is noteworthy that during prolonged exercise liver exports glutathione into plasma under the hormonal influence of glucagon (Li Li Jt, 1995: 18), thus contributing to the antioxidant natural defense of the organism.

Grassi *et al.* (2005) have shown that short-term administration of dark chocolate is followed by significant insulin sensitivity in healthy individuals and may improve the use of glucose by the peripheral tissues. Results of Chen *et al.* (1996) indicated that after chocolate bar ingestion glucose levels are increased significantly up to 5.82 $mmol.l^{-1}$ at the 15^{th} min of moderate exercise and maintained at 4.92 $mmol.l^{-1}$ until the 30^{th} minute. Blood lactate levels have shown beneficial changes during exercise and recovery, which corresponds to our findings. Increased lactate levels up to 18.7 $mmol.l^{-1}$ in top swimmers in response to maximal test have been reported by Grancharov (1997). During submaximal exercise lower accumulation of lactate is considered a favourable metabolic change related to a higher level of training status of the athletes. Lactate has been demonstrated by electron spin resonance (ESR) spectroscopy to scavenge hydoxyl and superoxide radicals. It has been speculated that lactate might reduce oxidised species by acting as hydrogen donor preventing the oxidation of glutathione (Deaton & Martin, 2004: 2). The mechanisms that could explain the effects of flavinols and other antioxidants on lactate metabolism need to be elucidated.

In conclusion, short-term dark chocolate administration might, via mechanisms of reducing ROS production, increasing insulin sensitivity and mobilising liver glutathione to supply muscle tissue, modulate and benefit metabolic changes in uric acid and lactate in response to exercise-induced oxidative stress.

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