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EFFECTS OF SUPPLEMETATION WITH RED GRAPE SKIN POLYPHENOLIC EXTRACT AND INTERVAL SWIMMING TEST ON THE BLOOD ANTIOXIDANT STATUS IN HEALTHY MEN

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Abstract

Introduction: Grapes *Vitis vinifera* and red wine are rich sources of polyphenolic substances which may potentially enhance the antioxidant capacity of plasma in humans.

The aim of the study: The study was aimed at the assessment of the effects of the alcohol–free red grape skin polyphenolic extract on the blood antioxidant status in healthy male subjects subjected to interval swimming test.

Materials and methods: Fourteen physical education students randomized to two groups: the control group (n=5) and the experimental group (n=9) supplemented with an alcohol-free polyphenolic extract from the skins of *Vitis vinifera* red wine grapes. One capsule containing 390 mg of red grape skin extract was administered three times daily for 6 weeks. The students participated in the interval-type swimming test (free-style with moderate to high intensity) at two occasions i.e. before the onset of the study (1st trial) and then once more after 6 weeks (2nd trial). In venous blood samples taken at rest, immediately after completion of interval swimming test and after 1h recovery the activities were measured of antioxidant enzymes: glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), level of glutathione (GSH). Creatine kinase (CK) activity and concentrations of uric acid (UA), lactate (LA) and total antioxidant status (TAS) were measured in plasma.

Results: A marked tendency was observed toward lower, as compared to values recorded in the 1st trial, pre- and post-exercise CK activities after a 6-wk -long supplementation with red grape skin extract (F=4.01; P=0.056 by 3-way ANOVA). A 6 wk-long supplementation had an insignificant modification of antioxidant enzyme (SOD, CAT, GSH-Px, GR) activities, concentrations of non-enzymatic antioxidants (GSH, UA) and total antioxidant status (TAS) were observed. The beneficial effect of an alcohol-free red wine grape extract on hemodynamic parameters. A comparison of swimming speeds and heart rates (HR) attained by subjects during interval swimming tests has evidenced that during the 2nd trial the participants from the supplemented group were able to swim faster at the final 50 m repeat (improvement from 1.18±0.03 to 1.31±0.11 $m \cdot s^{-1}$) at HR's slightly lower from those recorded during the first trial. Mean swimming speed attained by participants from the control group at the final 50 m distance during the 2nd trial was also higher (improvement from 1.22 to 1.29 $m \cdot s^{-1}$), however this was accompanied with an increase in HR from 184.6 to 187.6 $beats \cdot min^{-1}$.

Conclusions: The above presented results indicate that the supplementation with the alcohol free red wine grape polyphenolic extract may influence on attenuation of the post-exercise release creatine kinase (CK) into the circulation. Supplementation with this extract seems to be promising for the improvement of the hemodynamic status of subjects subjected to physical loads.

Key words: *red wine grape polyphenolic substances, antioxidant enzymes, total antioxidant status (TAS), creatine kinase (CK), physical exercise*

Introduction

Oxygen consumption during intense physical work may increase as much as 20-fold which is associated with a markedly increased production of oxygen radicals (1). Reactive oxygen species (ROS), including hydroxyl radicals, superoxide radicals, hydroperoxides or aldehydes are known to be toxic, mutagenic and carcinogenic to cells. It has been suggested that an increased production of ROS plays an important role in exercise-induced muscle damage (1-3). In spite of the intervention of the cell's antioxidant defense system, free radical-mediated lipid peroxidation can lead to the loss of integrity of cell membranes and to tissue damage.

Inactivation and removal of reactive oxygen species (ROS) depend on the capacity of the antioxidant defense system determined by a dynamic interaction between antioxidant enzymes (superoxide dismutase - SOD, EC 1.15.1.1, glutathione peroxidase - GSH-Px, EC 1.11.1.9), catalase - CAT, EC 1.11.1.6, glutathione reductase - GR, EC 1.6.4.2), and the non-enzymatic antioxidants which include low-molecular weight molecules such as tocopherol (vitamin E), ascorbic acid (vitamin C), beta-carotene, glutathione - GSH, and uric acid.

It has been suggested that exercise-induced muscle damage could be prevented through the supplementation of dietary antioxidants (4). Antioxidant properties

Table 1. *Basic characteristics of the subjects (n=14)*

Variable	First trial		Second trial	
	Control Group (n=5)	Supplemented group (n=9)	Control Group (n=5)	Supplemented group (n=9)
Age (years)	21.0 (0.5)	21.6 (0.7)	21.0 (0.5)	21.6 (0.7)
Height (m)	180.3 (6.3)	182.3 (8.2)	180.3 (6.3)	182.3 (8.2)
Body mass (kg)	78.5 (8.9)	77.3 (8.5)	78.3 (8.3)	77.1 (8.8)
BMI ($kg \cdot m^{-2}$)	23.8 (0.6)	23.7 (0.9)	23.5 (0.5)	23.4 (0.9)

have also been evidenced in red wine grapes (*Vitis vinifera*) and red wines. Many studies have confirmed that grapes *Vitis vinifera* and red wine are rich sources of polyphenolic substances, that are usually subdivided into two groups, the flavonoids (quercetin, catechins, flavonols, anthocyanidins) and nonflavonoids (phenolic acids and resveratrol). These substances may potentially enhance the antioxidant capacity of plasma in humans as they behave as scavengers of ROS, as well as metal chelators and enzyme modulators (5-7). Resveratrol (3,5,4'-trihydrostilbene), a prominent representative of polyphenols found in the skins of red grapes and in red wine, has a variety of bioactivities associated with health promotion (8). Other human dietary sources of resveratrol are peanuts and peanut butter. The polyphenolic structure of this stilbene polyphenol confers antioxidant activity and may prevent oxidative stress-induced cellular damage (9,10). Resveratrol is also known as a potent antiarrhythmic agent with cardioprotective properties associated with its antioxidant activity and upregulation of nitric oxide (NO) production (11). This natural polyphenolic phytochemical as a chemoprevention agent has been shown to inhibit tumor initiation and the growth of cancerous cells through increased apoptosis, to reduce inflammatory processes by inhibition of prostaglandin production, cyclooxygenase-2 activity and to prevent the progression of fungal infection (9).

Numerous previous studies have been dedicated to the assessment of the benefits from antioxidant supplementation in physically active individuals and professional athletes (4,12). To our knowledge, there are no published reports on the combined effect of the exercise and supplementation with polyphenols derived from the skins of red wine grapes on the antioxidant status. The objective of the present study is to examine the effects of the alcohol–free red grape skin polyphenolic extract on the blood antioxidant status in healthy male subjects subjected to interval swimming test.

Methods

Subjects

Fourteen nonsmoking male physical education students aged 21.0±0.6 yr volunteered to take part in this study. All subjects were informed of the purpose and the nature of the study before giving their written consent to participate in the experiment, which had been approved by the Ethics Committee of the Academy of Physical Education. The basic characteristics of the subjects is presented in Table 1. None of the participants of the study was following a special diet or taking any drug known to affect antioxidant status of the blood.

Exercise protocol

The participants were randomly assigned into two groups: 1- the control group (n=5) and 2- the experimental group (n=9) supplemented with an alcohol-free polyphenolic extract from the skins of *Vitis vinifera* red wine grapes (Andean Medicine Centre Ltd. UK). The supplement presented in soft gelatinous capsules was administered at a dose of 1 capsule containing 390 mg of red grape skin extract three times daily for 6 weeks. The phenolic composition of the extract, as declared by the producer, was 188 $mg \cdot g^{-1}$ of polyphenols (catechin, gallic acid, quercetin, trans-resveratrol, cis-resveratrol) and 35 $mg \cdot g^{-1}$ of anthocyanidins (malvidin, peomidin, petunidin, delphinidin, cyanidin). The students participated in the interval-type swimming test (free-style with moderate to high intensity) at two occasions i.e. before the onset of the study (1st trial) and then once more after 6 weeks (2nd trial).

The interval freestyle (front crawl) swimming test consisted of six repeats of 50m with the first distance at 70-75% effort and subsequent 50m repeats with progressively increasing velocity with 10-20 seconds rest between swims and the last swim as a maximal effort. The heart rate (HR) was monitored before the start of the test and at the end of each distance by using Sport Tester Polar S610i (Polar Electro Oy, Finland) and the times on each repeat were measured by a stopwatch.

Venous blood samples were drawn into heparinized test tubes from the antecubital vein at rest, immediately after completion of interval swimming test and after 1h of passive recovery.

Analytical procedures

Fresh whole blood samples were immediately assayed for reduced glutathione (GSH) by a colorimetric method (13) with 5,5'-dithiobis-2-nitrobenzoic acid and the activity of GSH-Px by using commercial kit (RANSEL RS505, Randox,UK). The remaining blood was centrifuged for 10 min at 1,000 g at 4°C to sepa-

rate plasma and erythrocytes. The erythrocytes were washed three-times with cold (4°C) saline and kept frozen at - 20°C, for not longer than 2 weeks, until being assayed for activities of antioxidant enzymes, i.e. SOD using a commercially available RANSOD SD125 kit (Randox, UK), CAT by the method of Aebi (14) and GR according to Glatzle et al. (15). The activity of CAT was expressed as the rate constant (k) of a first order reaction of hydrogen peroxide decomposition related to the hemoglobin (Hb) content ($k \cdot gHb^{-1}$). One GR activity unit was defined as the reduction of 1μmol of oxidized glutathione (GSSG) per minute, monitored by a decrease in absorbance at 340 nm due to oxidation of NADPH consumed for reduction of GSSG. The activities of all antioxidant enzymes were measured at 37°C and expressed per 1 g of hemoglobin as assesed by a standard cyanmethemoglobin method using a diagnostic kit (HG980; Randox, UK).

Fresh plasma samples were assayed for activities of creatine kinase (CK, EC 2.7.3.2) at 37°C using commercial diagnostic kits (Randox, UK). The plasma concentration of lactate (LA) was assessed at 30°C by commercially available enzymatic, colorimetric kit (Randox, UK). The level of the Total Antioxidant Status (TAS) in fresh plasma samples was assayed using commercial diagnostic kits (Randox, UK).

Statistical analysis

Mean and (±SD) were calculated for all variables. All data were tested for homogeneity of variances using the Levene's test and then analyzed using a 3-way analysis of variance (ANOVA) for repeated measures, followed by the Bonferroni test. All statistical analyzes were performed using STATISTICA 5.0 (StatSoft, Inc. 1995) software. The level of significance was set at $p<0.05$.

Results

The obtained results are presented in Tables 1 to 3. Basic characteristics of participants is presented in Table 1. No between-group differences were found in somatic parameters recorded in both trials.

The results of biochemical analyses are presented in Tables 2 and 3. Supplementation with red grape skin extract appeared not to affect significantly the activity of SOD. There was only a tendency towards slightly higher SOD activities to occur immediately post-exercise. Despite the lack of significant differences between the pre- and post exercise values a repeated measures 3-way ANOVA revealed a significant ($F = 4.47$; $p<0.05$) -effect of exercise.

Similarly, no significant, exercise-induced changes in CAT and GSH-Px activities were found in both the control and supplemented groups in each trial. There was only a tendency toward slightly higher CAT and GSH-Px activities immediately post-test. Interestingly, the contrary trends in GSH-Px activities were observed between 1st and 2nd trial, i.e. an increase (significant in the case of resting values; $p<0.05$) in the control group and a decrease – in the experimental group receiving the supplement. In both groups of subjects the post-exercise changes in GR activities recorded in both trials were small and insignificant.

No significant effect was found of the supplementation and exercise on concentrations of GSH in erythrocytes.

There was a tendency toward delayed increase in UA concentration in plasma during the recovery period from the interval swimming test, as reflected by a significant exercise- effect ($F=24.04$; $p<0.05$ by 3-way ANOVA). Ingestion of the red grape skin extract led to a significant ($p<0.05$; by 3-way ANOVA) decrease in the resting plasma concentrations of UA in subjects from the supplemented group.

Plasma concentrations of TAS were slightly higher after interval swimming test, the effect of exercise was significant ($F=27.05$; $p<0.001$ by 3-way ANOVA), but the effect of the supplementation did not reach the significance level.

The interval swimming test induced changes in plasma activity of CK, the results are presented in Table 3. A significant increase in CK activities was observed immediately after completion of the swimming test, and a significant exercise-effect ($F=27.19$; $p<0.0001$) was revealed by 3-way ANOVA. Moreover, a marked tendency was observed toward lower, as compared to values recorded in the 1st trial, pre- and post-exercise CK activities after a 6-wk -long supplementation with red grape skin extract, and the effect appeared to be close to the significance level ($F=4.01$; $p=0.056$ by 3-way ANOVA). It is noteworthy that all CK activities were within the reference range for men (24-195 $U \cdot l^{-1}$) (16).

The results of plasma lactate (LA) determinations recorded during interval swimming test are presented in Table 3. The post-test lactate concentrations were found to be significantly ($p<0.001$) higher than the pre-test values. A significant main effect of exercise ($F=2048.3$; $p<0.001$) on LA level was found by 3-way ANOVA.

The swimming speeds and post-test heart rates (HR) (Table 4) recorded during all repeats of the interval swimming test are presented in Table 4. With the aim of revealing whether supplementation with the red grape extract could affect performance, we have calculated the differences between swimming speeds achieved at individual repeats of the interval swimming test during the 2nd and the 1st trials. Mean results calculated separately for the control and the supplemented groups are presented in Fig. 1. The significant differences ($p<0.05$) in swimming speeds between the control group and the experimental group supplemented with the red grape skin extract were measured at the five distance of individual 50m repeats of the interval freestyle swimming.

Table 2. *Mean (±SD) pre- and post-test activities of the antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and the plasma Total Antioxidant Status (TAS) in the control and the experimental group supplemented with red grape skin extract*

Variable	Control group						Supplemented group					
	First trial			Second trial			First trial			Second trial		
	rest	after test	1h post	Rest	after test	1h post	Rest	after test	1h post	rest	after test	1h post
SOD ($U \cdot gHb^{-1}$)	1043.4 (691.9)	1185.1 (683.0)	907.1 (420.2)	1182.7 (140.9)	1375.4 (158.8)	1467.1 (197.2)	1199.8 (386.1)	1240.1 (395.9)	1257.9 (527.6)	1176.2 (128.5)	1359.4 (219.9)	1478.3 (364.7)
CAT ($s^{-1} \cdot gHb^{-1}$)	177.2 (33.5)	185.2 (37.3)	196.0 (46.6)	207.9 (24.6)	198.2 (28.8)	200.8 (19.2)	207.9 (32.8)	209.9 (29.4)	216.5 (39.2)	187.2 (33.5)	214.6 (42.7)	230.9 (51.7)
GSH-Px ($U \cdot gHb^{-1}$)	27.2 (4.13)	30.9 (6.6)	34.9 (7.2)	43.1# (5.6)	49.3 (12.7)	44.3 (5.8)	35.9 (3.9)	42.1 (9.8)	38.1 (6.6)	27.9 (8.7)	31.0 (10.8)	34.9 (9.5)
GR ($U \cdot gHb^{-1}$)	23.0 (4.3)	21.9 (2.7)	24.3 (4.3)	23.3 (7.9)	26.0 (3.2)	23.6 (9.2)	23.7 (2.7)	23.0 (2.8)	24.2 (4.6)	25.8 (6.3)	26.7 (4.7)	25.1 (7.1)
TAS (μmol/L)	798.1 (166.1)	891.8 (97.9)	959.3 (139.5)	814.7 (132.0)	972.5 (131.2)	053.6 (100.7)	773.9 (55.3)	835.2 (64.0)	908.8 (132.4)	811.4 (114.9)	878.7 (141.8)	1029.9 (213.9)

Note: Significantly (#p<0.05) different from the corresponding value in 1st trial

Table 3. *Mean (±SD) pre- and post-test concentrations of blood glutathione (GSH), plasma lactate (LA) and uric acid and activities of plasma creatine kinase (CK) and lactate dehydrogenase (LD) in subjects from the control and the experimental group supplemented with red grape skin extract*

Variable	Control group						Supplemented group					
	First trial			Second trial			First trial			Second trial		
	Rest	after test	1h post	Rest	after test	1h post	Rest	After test	1h post	Rest	after test	1h post
GSH ($\mu g \cdot gHb^{-1}$)	1.8 (0.2)	1.7 (0.4)	1.8 (0.4)	2.2 (0.3)	1.9 (0.5)	2.3 (0.7)	2.3 (0.2)	1.8 (0.2)	1.5 (0.2)	2.4 (0.3)	1.8 (0.1)	2.3 (0.4)
Uric acid ($mg \cdot dl^{-1}$)	2.8 (0.7)	3.2 (0.7)	3.9* (0.7)	4.7 (1.1)	5.4 (0.8)	6.5* (0.5)	4.0# (0.9)	4.9 (1.2)	5.9 (1.8)	3.5# (0.7)	3.9 (0.8)	4.4 (0.5)
CK ($U \cdot l^{-1}$)	142.3 (52.4)	186.0** (57.2)	167.2* (40.8)	172.7# (59.6)	208.3* (79.2)	180.5 (60.2)	135.2 (59.7)	168.2* (75.1)	151.1 (74.8)	101.2# (28.3)	127.3* (31.9)	116.2 (33.8)
LA ($mmol \cdot l^{-1}$)	2.1 (0.4)	14.5** (0.9)	2.8 (0.6)	3.4 (0.6)	12.9** (0.7)	2.8 (0.6)	3.4 (0.6)	13.8** (0.7)	3.2 (0.9)	3.2 (0.5)	13.7** (1.2)	2.9 (0.6)

Note: Significantly (*p<0.05, ** p<0.001) different from the resting value; Significantly (#p<0.05) different from the corresponding value in 1st trial.

Table 4. *Swimming speeds and mean HR's recorded at each of six 50 m repeats of interval freestyle swimming test in the control and the experimental group supplemented with red grape skin extract*

Repeat No.	Control group				Supplemented group			
	1th trial		2nd trial		1th trial		2nd trial	
	V ($m \cdot s^{-}1$)	HR	V ($m \cdot s^{-}1$)	HR	V ($m \cdot s^{-}1$)	HR	V ($m \cdot s^{-}1$)	HR
1	1.08 (0.03)	147.2 (15.8)	1.10 (0.02)	155.0 (11.7)	1.07 (0.09)	142.0 (10.8)	1.12 (0.05)	152.0 (13.8)
2	1.07 (0.03)	157.4 (16.0)	0.88 (0.48)	164.2 (12.1)	1.12 (0.07)	158.6 (9.3)	1.10 (0.07)	161.1 (9.6)
3	1.14 (0.04)	167.0 (14.1)	1.11 (0.08)	165.6 (19.2)	1.15 (0.09)	168.8 (8.6)	1.15 (0.07)	171.2 (5.3)
4	1.20 (0.07)	170.0 (14.1)	1.13 (0.11)	173.0 (9.8)	1.19 (0.06)	170.8 (8.6)	1.16 (0.09)	170.0 (7.4)
5	1.22 (0.12)	180.0 (9.7)	0.99 (0.48)	179.0 (8.8)	1.20 (0.15)	175.7 (10.0)	1.19 (0.08)	173.8 (8.9)
6	1.22 (0.02)	184.6 (9.5)	1.29 (0.18)	187.6 (9.1)	1.18 (0.03)	186.7 (12.4)	1.31 (0.11)	185.3 (8.6)

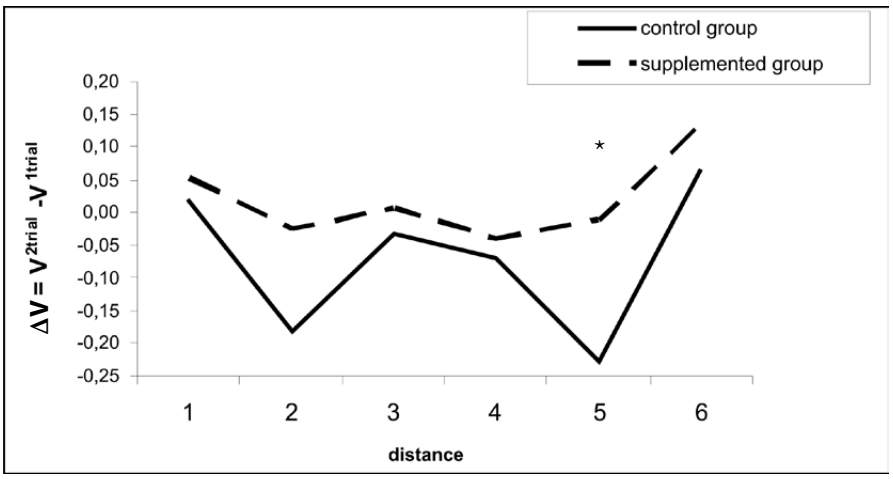

Figure 1. *The differences in swimming speeds* ($\Delta V = V^{2\,trial} - V^{1\,trial}$) *attained at individual 50m repeats of the interval freestyle swimming test in control group and the experimental group supplemented with the red grape skin extract*
Note: Significantly (*p<0.05) different from the corresponding value in the supplemented group

Discussion

Numerous previous studies have evidenced that the efficiency of the antioxidant defense system in humans depends on endogenous production of antioxidants and on adequate dietary vitamin and micronutrient intake (17). It is well established that several naturally occurring foods, such as skins and seeds of red wine grapes contain a very high concentration of flavonoid and nonbioflavonoid polyphenols, which are potent antioxidants. The antioxidant capacity of red wine and alcohol-free red grape skin extracts has been shown in different *in vitro* and *in vivo* systems (18). Polyphenols act as antioxidants by the hydrogen-donating property of their hydroxyl groups (6,19,20). Apart from their capacity to scavenge free radicals, polyphenols may prevent hydroxyl radical formation through the Haber-Weiss/Fenton reactions, due to their metal-chelating properties (21). It was evidenced that consumption of red wine increases the antioxidant capacity of serum (7). Much less is known about the potentially beneficial effects of the extract prepared from the grape *Vitis vinifera* on the antioxidant status of physically active individuals, both men and women, which has motivated us to undertake our investigation.

The most interesting finding of the present study was that a 6 week-long consumption of the red grape skin extract resulted in a significant decrease (main effect) in CK activities and a significant decline in resting CK activity in the plasma (Table 3). The release of creatine kinase (CK), an integral part of the ATP/creatine phosphate energy system of muscle, into circulation, as evidenced by an increase in activity of this enzyme in the serum or plasma, is considered the most sensitive indicator of muscle injury (22). There is a substantial evidence that exercise-induced oxidative stress may play a pivotal role in both the initiation and progression of muscle cell membrane disruption (23). One of the major factors responsible for the increase in the permeability of plasma membranes, which results in a "leakage" of intracellular proteins into the circulation, is peroxidation of membrane lipids, markedly enhanced during strenuous exercise (24). In the study reported here, post-exercise CK activities recorded in both groups during both trials were significantly higher from the resting values. It is worth noting that CK activities recorded during the 1st trial in both groups of participants were very close to each other. Interestingly, as compared to the 1st trial, CK activities recorded during the 2nd interval swimming test, were higher in the control group, while the contrary trend toward lower values was observed in subjects receiving the red grape skin extract. Most likely, the antioxidant capacity of this extract played an important role in maintaining the integrity of the cell membrane post-exercise and limiting CK release from skeletal muscle. In this regard, our results are in accordance with those reported by Morillas-Ruiz et al. (25) for the polyphenol–supplemented individuals subjected to intense exercise.

It should be stressed, however, that although the antioxidant properties of most of the phenolic components of red wine grapes have been shown *in vitro*, much less is known about the antioxidant potential of individual components *in vivo* (18,26,27). Moreover, in most studies the antioxidant effects of the specific red wine constituents appeared not to be secondary to changes in plasma concentrations of antioxidant vitamins (28,29).

Although the results of our study have indirectly evidenced the antioxidant potential of the red grape skin extract in attenuating signs and symptoms of exercise-induced skeletal muscle injury in young physically active men, little direct influence on their blood antioxidant status, as mostly insignificant modification of antioxidant enzyme (SOD, CAT, GSH-Px, GR) activities, concentrations of non-enzymatic antioxidants (GSH, UA) and total antioxidant status (TAS) were observed. Post-exercise increases in antioxidant enzyme activities appeared also to be relatively small and mostly insignificant, although the main-effect of exercise appeared to be significant, but only in the case of SOD.

The discrepancies in findings among studies suggest that type and intensity of exercise may affect the response of the blood antioxidant defense system. In a recent study of Nikolaidis et al. (30) with adolescent swimmers aged 9-11 y subjected to an interval swimming test (12 bouts of 50 m at a pace of 70-75 % of the participant's maximum 50 m speed) significant increases in CAT activity, TAS as well as significant decreases in GSH were reported. Although directions of post-exercise changes recorded in our study were similar, i.e. the red blood cell activities of SOD, CAT and GSH-Px also tended to increase in response to the interval swimming test; the magnitude of changes was much less (Table 2). Similarly, the post-exercise decline in GSH concentration observed in both groups of participants has not reached the level of significance. As expected, the plasma concentrations of urate tended toward delayed increase during the recovery period, which appeared to be significant only in the control group (Table 3). Interestingly, in subjects supplemented with the red wine grape extract the resting plasma concentrations of uric acid, recorded during the 2nd trial, were significantly lower than the baseline values. These observations suggests that interval swimming test, as applied in our study, resulted only in subtle changes in the capacity of the antioxidant defense system in the blood.

Among the most promising health implications related to consumption of flavonoids and polyphenolic components derived from red wine grapes are their antiatherogenic and cardioprotective effects (8,11,31,32). Such a suggestion originated from epidemiologic observations among inhabitants of Southern France who have a very low mortality rate due to coronary heart disease (CHD) despite a high-fat diet, little exercise, and a wide-spread cigarette smoking. This phenomenon, known as French Paradox, is attributed to a relatively high intake of red wine.

Several experiments performed on animal models have evidenced a strong cardioprotective effect of red wine polyphenols. Mokni et al. (10), who studied selected hemodynamic parameters, such as heart rate (HR) and developed pressure (DP) in isolated hearts from control and resveratrol treated rats, observed a marked improvement in post- ischemic indices of myocardial function after 7 day-long intra-peritoneal administration of this polyphenol (but not after an acute treatment), which could be only partly explained by its antioxidant properties. Another group (33) who examined the effect of resveratrol on ventricular function of rat hearts after ischemia–reperfusion (I/R)–induced injury observed also a dramatic improvement in post-ischemic ventricular functional recovery. Moreover, these cardioprotective effects were correlated with both the antioxidant activity of resveratrol and up-regulation of nitric oxide (NO) production. Previous studies (34) with the use of human umbilical vein endothelial cells exposed to an alcohol-free red wine grape extract evidenced augmented release of NO from endothelial cells in a concentration-dependent manner, which may be attributed to an over expression of endothelial NO synthase (eNOS) mediated by red wine polyphenols.

Direct effects of red wine or alcohol-free wine extracts on endothelial cells have been reported by several authors, who concluded that NO release induced by direct action of some anthocyanins and other polyphenols present in red wine may be involved in the increase in coronary flow vascular reserve, as observed after consumption of red wine in both healthy human volunteers and patients with CAD (8,35,36).

The results of our study seem to support indirectly these observations on the beneficial effect of an alcohol-free red wine grape extract on hemodynamic parameters. A comparison of swimming speeds and heart rates (HR) attained by our subjects during interval swimming tests (Table 4, Fig.1) has evidenced that during the 2nd trial the participants from the group supplemented with the red grape skin extract were able to swim faster at the final 50 m repeat (improvement from 1.18±0.03 to 1.31±0.11 $m \cdot s^{-1}$) at HR's slightly lower from those recorded during the first trial. Mean swimming speed attained by participants from the control group at the final 50 m distance during the 2nd trial was also higher (improvement from 1.22 to 1.29 $m \cdot s^{-1}$), however this was accompanied with an increase in HR from 184.6 to 187.6 beats/min. Moreover, although all students enrolled for the experiment were from the same study year, which implies that their weekly activity profile was similar, those from the control group had generally worse results in swimming speeds (Fig.1) recorded during the 2nd trial (except for the final 50 m distance). Swimming speeds attained during the 2nd trial by individuals supplemented with the red grape skin extract were not worse than those recorded before starting supplementation (1st trial), and a marked improvement was observed at the last 50 m distance. Of importance is the fact that there was a marked tendency toward lower HR values over the last three 50 m repeats despite unchanged (on 4th and 5th repeats) or visibly higher swimming speed attained at the last 50 m distance during the 2nd interval swimming test.

Taking together, the differences in the response to physical loads, as those applied in the interval swimming test, seem to support the hypothesis of the beneficial effects of supplementation with the alcohol free red wine grape extract on maintenance of integrity of the muscle cell membranes and attenuation of the post-exercise release of the muscle cell enzyme (CK) into the circulation, despite only a minor impact on the capacity of the blood antioxidant defense system. Supplementation with red wine grape polyphenolic extract seems to be promising for the improvement of the hemodynamic status of subjects subjected to physical loads.

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THE CHANGES IN CUTANEOUS MICROCIRCULATION IN JUDO ATHLETES BEFORE THE PREPARATION PERIOD AND IN THE COMPETITION PERIOD

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Abstract

Introduction: Circulatory adaptation to physical exertion is an important determinant of athletes' practising capability. Little is known about the reaction of the cutaneous microcirculation in athletes at various stages of the training cycle. That's why the aim of this study was to explore, whether chosen parameters of the cutaneous microcirculation in judo athletes, measured using laser Doppler flowmetry, change between the start of the training (non-training period) and the competitions phase (competition period).

Material and methods: The study was performed in 11 men, judo athletes and Polish team champions 2006, mean age 24.18±8.84 yr. The microcirculation was measured using Doppler laser flowmeter Periflux 4001 (Perimed, Sweden). The technique applied in the instrument uses the laser light of wavelength 780 nm. It measures the blood flow which is a product of number of moving erythrocytes in the given tissue times their mean speed. The test was performed in horizontal position (on the back), in constant surrounding temperature of 21°C±1.2°C, air humidity 40-60%, after ca. 20 minutes of adaptation. The optode was placed on the skin of the back of the hand between the first and second metacarpal bones using special both sides adhesing ring. Rest flow, hyperaemic, hyperthermic and orthostatic reactivity of skin microcirculation and maximal minute oxygen uptake ($\dot{V}O_2max$), were evaluated. All parameters were recorded twice: immediately before the preparation period and in the competition period – before the Polish Championship.

Results: Beside biological zero, all other measured parameters were statistically significant higher in the competition period (rest flow – 28.64±5.28 PU; biological zero – 2.94±0.37 PU; perfusion at the peak intensity of the signal during the reactive hyperemia –130.61±22.96 PU; perfusion at the peak intensity of the signal during the thermal hyperemia – 265.99±39.89 PU; orthostatic reactivity – 48.51%; $\dot{V}O_2max$ – 58.05±15.07 ml·kg·min^{-1}), compared with the preparation period (rest flow – 18.67±3.88 PU; biological zero – 2.84±0.42 PU; perfusion at the peak intensity of the signal during the reactive hyperemia – 96.57±24.42 PU; perfusion at the peak intensity of the signal during the thermal hyperemia – 192.99±34.32 PU; orthostatic reactivity – 43.33%; $\dot{V}O_2max$ – 47.12±7.66 ml·kg·min^{-1}). The frequency analysis showed smaller oscillations of the sympathetic system and also in the cardiac frequency and increased activity of the endothelium in the competition period.

Conclusions: Chosen parameters of the cutaneous microcirculation in judo athletes, measured using laser Doppler flowmetry, change between the start of the training (non-training period) and the competitions phase (competition period). In the competition period the capacity and efficiency of the cutaneous vascular bed increases.

Key words: *skin microcirculation, laser Doppler flowmetry, physical training.*

Introduction

Circulatory adaptation to physical exertion is an important determinant of athletes' practising capability. When a relatively big mass of muscle is being regularly activated, the circulatory parameters improve not only in the locally active muscles but also for the whole organism. Adaptive changes refer also to the cutaneous vascular bed (1,2). Many authors observed that a systematic physical training stipulates the vasodilation in the cutaneous microcirculation both in animals and men (3-5). The increase of the vessels' diameter is probably mediated by nitric oxide (NO) which is produced in the endothelium. O'Sullivan observed increased metabolic turnover of the endothelium and improved action of the vasodilative factors after five weeks of moderately intensive training (6). Wang noticed that physical exertion results in more efficient function of the endothelium, while forced immobility led to decreased concentrations of NO metabolites in blood (7). Clarkson et al. proved that aerobic physical training enhances endothelium-dependent vasodilation, which was demonstrated by increased value of the hyperaemic reaction to administration of the endothelium-dependent vasodilator after a finished cycle of aerobic and anaerobic exercises (8).

Little is known about the reaction of the cutaneous microcirculation in athletes at various stages of the training cycle. That's why the aim of this study was to explore, whether chosen parameters of the cutaneous microcirculation in judo athletes, measured using laser Doppler flowmetry, change between the start of the

training (non-training period) and the competitions phase (competition period).

Material and methods

Subjects

The study was performed in 11 men, judo athletes and Polish team champions 2006, mean age 24.18±8.84. All of them had normal blood pressure and the body mass index (BMI). A characteristic of the tested group is presented in table 1.

The study was approved by the Bioethics Committee of the Medical Council in Opole (Resolution No 132 on 24.11.2005).

Measurement of skin blood perfusion

The test was performed in horizontal position (on the back), in constant surrounding temperature of 21°C±1.2 °C, air humidity 40-60%, after ca. 20 minutes of adaptation. The microcirculation was measured using Doppler laser flowmeter Perifluks 4001 (Perimed, Sweden). The technique applied in the instrument uses the laser light of wavelength 780 nm. It measures the blood flow which is a product of number of moving erythrocytes in the given tissue times their mean speed. The optode was placed on the skin of the back of the hand between the first and second metacarpal bones using special both sides adhesing ring. The flow was measured in conventional Perfusion Units score (PU), in proportion to the energy of the Doppler signal. 1 PU corresponds the voltage of 10 mV at the outlet (9). The all participants were asked not to take part in physical activities and to avoid products that influence the circulation (coffee, tea and Coca-Cola).

Rest flow (after 20 minutes of stabilization of the circulatory system in horizontal position), hyperaemic (after loosening the cuff of the manometer filled with air up to pressure exceeding the formerly measured systolic pressure by 50 mmHg), hyperthermic (after rising the optode's temperature up to 44°C, using the built-in heating module) and orthostatic reactivity (changing the position from horizontal to vertical) of skin microcirculation were evaluated. During the post-occlusive hyperaemic reaction the time to reach half maximal hyperaemia (TH1) was also measured as well as time to reach the maximal hyperaemia (TM), time to fall back to half maximal hyperaemia (TH2) and time from max hyperaemia back to baseline (TP). All parameters were recorded twice: immediately before the preparation period and in the competition period – before the Polish Championship.

Spectral analysis of laser Doppler signal

Frequency of signals received by means of the laser Doppler fluximetry between 0.01 up to 2 Hz during basic flow was also analyzed. In this range five groups were singled out: I – frequency band between 0.01-0.02 Hz; II – frequency band between 0.021-0.05 Hz; III – frequency band between 0.051-0.145 Hz; IV – frequency band between 0.15-0.5 Hz; V – frequency band between 0.51-2 Hz. In each band range there is a different factor which determines blood flow oscillation. I – shows vascular oscillations depending on the endothelium metabolic activity (ER); II – shows the effect of the sympathetic system on skin flow (SR); III – illustrates oscillations resulting from the arteriola basic systolic tonus which occurs due to discharges of particular myocytes forming a circular layer of the vessel muscle coat, this response is often referred to as myogenic and it is independent of the sympathetic system (MR); IV – breath frequency (BR); V – heart frequency (HR). A time constant of 0.03 s was selected, and every blood flow signal was taken at the frequency of 32 Hz (10,11). Apart from frequency, the signal power was also analyzed.

Table 1. *Morphologic and physiologic characteristic of the tested athletes*

before the preparation period / competition period							
Lp.	Age [years]	Height [cm]	Weight [kg]	BMI [kg·m^{-2}]	$\dot{V}O_2$max [l·min^{-1}]	$\dot{V}O_2$max [ml·kg^{-1}·min^{-1}]	HR [cycle·min^{-1}]
1.	23	175	78/76	25.5/24.8	4.33/6.88	54.9/88.3	60/60
2.	42	182	82/80	24.7/24.1	3.88/4.42	46.8/54.9	61/61
3.	40	178	75/74	23.6/23.3	3.74/4.43	49.2/59.9	60/59
4.	15	165	64/63	23.5/23.1	2.57/2.9	40.1/45.3	61/60
5.	20	168	62/61	21.9/21.6	3.71/4.76	59.8/76.7	66/60
6.	17	176	62/62	20/20	2.87/3.83	46.2/61.8	76/60
7.	24	182	85/81	25.6/24.4	3.34/3.52	37.1/39.2	61/60
8.	23	176	73/71	23.5/22.9	3.58/4.13	49.7/56.6	72/60
9.	22	187	86/84	24.5/24	3.46/3.89	38.4/43.2	72/60
10.	23	168	65/64	23/22.6	2.61/2.87	40.1/45.5	72/65
11.	23	165	64/62	23.5/22.7	3.58/4.36	56.0/67.1	68/70

The test of the maximal oxygen uptake ($\dot{V}O_2max$)

The maximal oxygen uptake ($\dot{V}O_2max$) was measured directly using the ergospirotest produced by Jaeger. The power test was of progressive nature and was performed on a cycloergometer with pedalling velocity of 60 rpm. The test was started at the load of 50W and increased every 3 min by another 50W until refusal to further exercise. Test was also stopped when increasing load did not evoke increase in VO_2.

Statistical analysis

The values of the examined parameters were presented as median ± standard deviation (SD). Statistic analysis was performed with the use of t-student test, assuming $p<0.05$ to be significant.

Results

The values of the physiological parameters obtained during the test of the aerobic power allowed to evaluate the aerobic capability of the tested athletes. Higher values of the maximal minute oxygen uptake ($\dot{V}O_2max$), both globally and per kg of the body mass were found during the competition period (4.18±1.08 $l \cdot min^{-1}$ and 58.05±15.07 $ml \cdot kg^{-1} \cdot min^{-1}$ respectively). In the non-training period the values were respectively 3.42±0.54 $l \cdot min^{-1}$ and 47.12±7.66 $ml \cdot kg^{-1} \cdot min^{-1}$. The values calculated per kg of the body mass were higher in the competition period and the difference was statistically significant ($p = 0.04$).

Basic flow recorded after 20 min adaptive period was significantly ($p < 0.001$) higher in the competition period (28.64±5.28 PU) than in the non-training stage (18.67±3.88 PU).

Biological zero in healthy individuals oscillates around zero or has insignificant values resulting from random movements of the blood cells. In the tested group its values were 2.94±0.37 PU in the competition period and 2.84±0.42 PU in the non-training period.

Post-occlusive hyperaemic reaction is the response of the vascular bed to opening of the sphygmomanometer's tourniquet after 4-min occlusion. Lower values of this parameter were characteristic for the non-training period (96.57±24.42 PU) while during the competition period a statistically significant ($p = 0.003$) increase occurred (130.61±22.96 PU). Mean values recorded in given training cycles are presented in the Fig. 1.

Mean values of time recorded in given training cycles are presented in the Fig. 2.

The vascular bed reacts intensely to thermal stimuli. Heating of the probe to 44°C resulted in increased blood flow up to 192.99±34.32 PU in the non-training period and to 265.99±39.89 PU in the competition period. The differences between the non-training and competition period were statistically significant ($p < 0.001$).

In orthostatic reaction decrease was greater and statistically significant ($p < 0.001$) in the competition period (48.51±1.47% compared to 43.33±1.85% in the non-training period).

The results of the analysis of the signal frequency and power when the basal flow was observed are presented in table 2.

Statistically significant differences occurred in the band of the cardiac rythm. In the competition period the number of cycles per minute was significantly ($p = 0.02$) lower than in the non-training period.

Regarding the respiration and myogenic rhythm bands as well as signal power in these bands no significant differences between the training stages were noted. However the signal power in the neurogenic band was significantly lower in the competition period

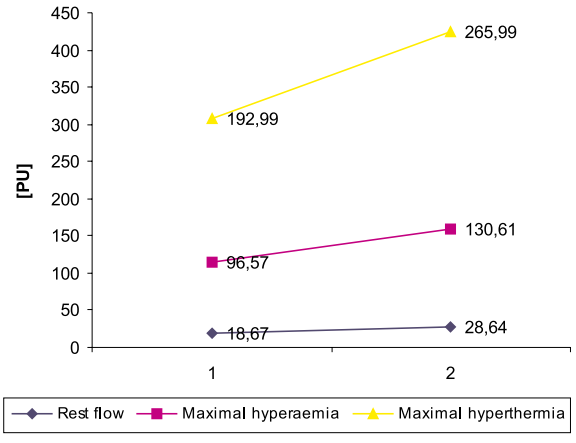

Fig.1. *Selected parameters of microcirculation in the non-training period (1) and competition period (2)*

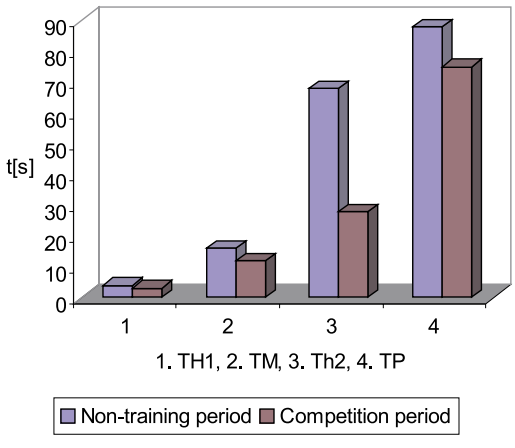

Fig.2. *Mean values of time recorded in given training cycles*

Table 2. *Mean values ($\bar{x}$) and standard deviation (SD) of the parameters analyzed in basal flow in given frequencies*

Parameter	preparation period		competition period	
	Frequency of signals [cycle·min^{-1}]	signal power	Frequency of signals [cycle·min^{-1}]	signal power
HR	66.42±5.67	0.51±0.5	61.56±3.22*	0.7±0.49
BR	13.32±1.9	0.39±0.18	12.65±1.15	0.57±0.37
MR	5.78±0.27	2.06±1.99	5.78±0.27	2.11±1.84
SR	1.98±0.27	2.85±1.04	1.98±0.27	0.76±0.36*
ER	0.9±0.0	2.21±1.29	0.9±0.0	4.05±1.62*

* statistically significant differences

compared to non-training period ($p<0.001$), whereas regarding the endothelial rhythm it was relevantly higher in the competition period ($p = 0.007$).

Discussion

The evaluation of the cutaneous microcirculation with the Doppler-laser flowmetry are mainly used in diagnostics of the cardio-vascular diseases and metabolic disorders (12,13). However it's being more and more frequently used in exploration of the changes occurring in the circulation under impact of the physical exercise. It is well known that regular physical activity reduces the systolic blood pressure (14), restores the decreasing with age fibrinolytic activity of the endothelium (15), increases the bioavailability of the NO (16-19), and improves organism's tolerance for the increases of the internal temperature (20).

So far there are no reports regarding the reaction of the cutaneous microcirculation in various stages of sport training, nevertheless it's known that regular physical activity, occurring in the preparatory and competition period, results in increased values of the parameters describing the vascular bed.

Many authors observed increased basal resting cutaneous flow in individuals who regularly practice aerobic exercise. Johnson et al. explain that fact with exertion adaptation within the autonomic nervous system; however it is not clear how the exercise would influence the vasoconstrictive and vasodilative nerves in the cutaneous vascular bed (20). Kraus et al. proved the increased serum concentration of vascular endothelium growth factor (VEGF; produced in the cells of the skeletal muscles and excreted to circulation) immediately after and 2 hours after exercise and suggested its correlation with increased blood flow (1).

Nevertheless majority of the authors explain the increased resting flows with improved function of the endothelium due to the physical exercise. The training-related increase in blood flow is probably one of the first factors to initiate adaptive changes of the endothelium. This should lead to increased shear stress and subsequently increased secretion of NO. Nitric oxide is probably one of the factors responsive for dilation of the blood vessels not only in the working muscles but also in the whole organism (2,21,22). Whereas Franzoni et al. reported comparable values of the basal flows in athletes and in non-practising individuals (16). Although this could be due to great age span in the tested group (22 – 72 years) while it is known that with growing age the activity of the endothelium decreases (17,23).

Author's own studies showed a significant increase in rest flow during a five months period of systematic training.

The capacity and efficiency of the cutaneous vascular bed is investigated with the use of evoked reactions. The stimulus is usually a change of temperature, occlusion or change of the body position. Own material shows that in the competition period the values of the maximal post-occlusive hyperaemic reaction, reaction to temperature, and orthostatic reaction are greater whereas the difference is statistically significant. These results are supported by other authors; Franzoni et al. found higher values of reaction to occlusion and temperature in 36 systematically practising sportsmen (24). Clarkson et al. proved increased values for hyperaemic reaction after forced program of aerobic and anaerobic exercises in a group of soldiers (8). Studies by Wang confirmed increased values of the hyperaemic reaction in 10 persons after 8-weeks training on a cycloergometer and their decline after forced lack of activity in the same persons (7).

O'Sullivan demonstrated higher values of the post-occlusive hyperaemia in physically active individuals compared to those with a sedentary way of life. Then the non-exercising group underwent a 5-weeks aerobic training, which resulted in an increase of the hyperaemic reaction comparable to the physically active group (6). The authors point to the fact of quick reversal of the positive, training-related changes in the peripheral vessels after cessation of systematic training (7). However decreased values of the evoked reactions are observed in patients with structural lesions of the arteriolae.

Time indices of the hyperaemic curve were also evaluated (TH1, TM, TH2, TP). After the post-occ-

lusive hyperaemia the blood flow should return to the resting values after 2 min. Results recorded during the study fitted in this range although the reaction in the competition period was faster. Prolongation of the time indices of the hyperaemic curve suggests disorders of the haemodynamics in peripheral circulation (25,26).

There's a considerable difference (40–50%) between skin flow in horizontal position and after standing up in healthy persons. After change of the position to erected the capillary hydrostatic pressure rises and circulation reacts with rapid vasoconstriction (using the axon reflex) thus avoiding excessive transfer of water to the extracellular space. During exercise the situation is similar. There is an increase of the capillary hydrostatic pressure and escape of the plasma from the intravascular space (27). The significant decrease of the blood flow that is observed in the competition period by 48%, 5% more than in the non-training period, may result from a more efficient reaction of the circulation due to systematic practising.

Evaluation of the oscillations of the signal frequency and power of the unstipulated blood flow showed no relevant differences in respiratory or myogenic rhythm between the given stages of the training. Substantial differences are present for the heart rhythm, which is much lower in the competition period. In this period the signal power regarding neurogenic oscillation was also substantially lower. Declined activity of the sympathetic nervous system due to exercise is well documented in literature (2,28).

Relevantly higher signal power in endothelium-dependent frequency range during the competition period that was recorded in the presented study results probably from better bio-availability and efficient endothelial release of NO due to regular training. Similar results were reported by Kvernmo et al. In the tested group of athletes these authors noted substantially higher oscillations at the frequency range of about 0.01 Hz compared to the control group (29).

Maximal oxygen uptake was another factor of differentiation between the two tested groups. The value of $\dot{V}O_2max$ expressed in $l \cdot min^{-1}$ as well as relative $\dot{V}O_2max$ calculated per kg of body mass was markedly higher in the competition period, which is also confirmed by other authors.

The presented results suggest a question, whether laser Doppler flowmetry can be used to evaluate the effects of training, but this problem requires further studies.

Conclusions

Chosen parameters of the cutaneous microcirculation in judo athletes, measured using laser Doppler flowmetry, change between the start of the training (non-training period) and the competitions phase (competition period). In the competition period the capacity and efficiency of the cutaneous vascular bed increases.

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PLASMA HORMONES, CERULOPLASMIN FERROXIDASE ACTIVITY AND TOTAL ANTIOXIDANT STATUS (TAS) CONCENTRATION IN REGULARLY MENSTRUATING WOMEN OF REPRODUCTIVE AGE WITH OVULATORY AND ANOVULATORY MENSTRUAL CYCLES

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Abstract

Introduction: Ovarian hormones apart from its contribution to reproduction are known to have a potent antioxidant activity. In animals ovariectomy induces oxidative stress which is alleviated by 17β-estradiol treatment. In women with disturbed ovary function increased lipid peroxidation, superoxide production and depressed antioxidant protection were found.

Aim of the study: This study was undertaken to evaluate plasma ceruloplasmin ferroxidase actvity, TAS and copper plasma levels in women with ovulatory and anovulatory menstrual cycles.

Methods: The prospective subjects monitored their basal body temperature (BBT) for 3 months before the study. Plasma ovarian hormone, T_3, T_4 and cortisol levels were determined twice per cycle – between days 5-8 and again between days 19 and 22 of the cycle. According to BBT pattern and plasma progesterone levels (>19 nmol · l^{-1}) 21 women were classified as ovulating and 16 as non-ovulating. Plasma TAS and copper levels were measured using Randox commercial kits. Plasma ceruloplasmin ferroxidase activity was assayed with o-dianisidnine dichloride. Plasma hormones were determined using radioimmunoassay methods.

Results: Plasma copper, T_3, T_4, and cortisol levels did not differ in ovulating and non-ovulating women. In both cycle phases plasma TAS levels and ceruloplasmin ferroxidase activity in non-ovulating women were higher than in ovulating ones ($p<0.02$ and $p<0.01$ for TAS and ceruloplasmin, respectively). No relationship was noted between TAS, ceruloplasmin ferroxidase activity and plasma hormone levels. In collected data from ovulating and non-ovulating subjects weak but significant correlations was noted between plasma TAS levels and ceruloplasmin ferroxidase activity ($r=0.242$, $p<0.04$).

Conclusion: It could be postulated that the elevation in plasma TAS levels and ceruloplamin ferroxidase activity in non-ovulating subjects possibly reflect compensatory response to increased oxidative stress due to disturbed ovarian hormone secretion. In addition, it seems feasible that at least in females ceruloplasim ferroxidase activity contributes to plasma antioxidant potential.

Key words: *plasma hormones, ceruloplasmin, TAS, menstrual cycle*

Introduction

Ovarian hormones apart from its contribution to reproduction are known to affect many metabolic processes as immunity, endothelial and islet function, bone mineralization and substrate utilization (1,2). In addition, it is well documented that estrogen has a potent antioxidant activity inhibiting lipid and LDL-cholesterol peroxidation and superoxide production *in vitro* (3,4). On the other hand, in animals ovariectomy induces oxidative stress in many tissues which is alleviated by 17β-estradiol treatment (5-8). The above-mentioned antioxidant estrogen action is probably due to the modulation of antioxidant enzyme expression and function (9-11). However, antioxidant action of ovarian hormones is not limited to estrogen, since progesterone has been demonstrated to protect against superoxide radical production (12).

Disturbed ovarian function as in PCOS increases lipid peroxidation, superoxide production and depresses antioxidant protection by decreasing reduced glutathione and total antioxidant status (TAS) in plasma (13,14). Furthermore, exercise-induced amenorrhea in female athletes possibly contributes to chronic oxidative stress causing adaptational elevation in erythrocyte glutathione peroxidase activity (15).

It is well documented that human body is protected against oxidative damage by both endogenous (SOD, catalase and glutathione system) and exogenous (nutrient-originated) antioxidants (16,17). In addition, blood plasma containing albumin, uric acid, bilirubin and thiols provides antioxidant protection against reactive oxygen species (ROS) detrimental action (18-21).

Numerous data have indicated that liver-originated ceruloplasmin despite contribution to iron homeostasis (22,23) acts as very effective antioxidant (24). In the presence of reduced glutathione, ceruloplasmin has been demonstrated to protect DNA against oxidative

stress (25). Moreover, ceruloplasmin has been noted to protect proteins against ROS-induced carbonyl formation (26). Antioxidant ceuloplasmin activity has been confirmed in isolated rat heart exposed to electrolysis-induced ROS action (27,28). According to Gutteridge and Quinlan (29) plasma ceruloplasmin together with iron-binding transferrin are the major plasma antioxidants although they represent no more than 4% of all plasma proteins.

It is well known that ceruloplasmin synthesis in the liver and it plasma levels are affected by ovarian hormones. In pregnant women as well as in women taking oral contraceptives ceruloplasmin plasma levels are higher than in controls due to estrogen-induced ceruloplasmin synthesis in the liver (30,31).

However, according to our best knowledge data concerning the effects of more subtle disturbances in ovarian hormone secretion on plasma ceruloplasmin ferroxidase activity and total antioxidant status (TAS) are not available.

Thus, this study was undertaken to evaluate plasma ceruloplasmin ferroxidase actvity and TAS levels in women with ovulatory and anovulatory menstrual cycles.

Materials and methods

Subjects

A total of 37 reproductive age women volunteered to participate in the study. All the women were healthy non-smokers. None of the participants were taking any medication on the regular basis. The prospective subjects monitored their basal body temperature (BBT) for 3 months before the study (32). Plasma ovarian hormone levels were determined twice per cycle – between days 5-8 and again between days 19 and 22 of the cycle. In 21 women with biphasic BBT pattern plasma progesterone concentrations exceeded 19 nmol $\cdot$ l^{-1} between days 19 and 22 of the cycle and they were classified as ovulating (33). In 16 women , the lack of increase in BBT was noted, and plasma progesterone concentrations between days 19 and 22 of the cycle was lower than 19 nmol $\cdot$ l^{-1}. Those participants were classified as non-ovulating. The participants were recommended to follow their habitual diet and lifestyle throughout the study. All the women gave their written consent before the study and the procedures were approved by the Ethics Commission at the University of Physical Education in Warsaw.

Analytical methods

Fasting venous blood was drawn into lithium heparin tubes using disposable syringes and needles under aseptic conditions between 8:30 and 9:00 a.m. Erythrocytes were separated by centrifugation (15 min/4000 rpm) and plasma was stored at – 70 ^{o} C until analysis.

Plasma copper concentrations were determined at 580 nm. Plasma total antioxidant status (TAS) was assayed with ABTS at 600 nm. Both copper and TAS plasma levels were determined using Randox commercial kits (Randox Laboratories, United Kingdom) Intra- and inter-assay coefficients of variation (CV) for copper were 4.2% and 5.8%, for TAS 4.5% and 6.3%, respectively. Plasma ceruloplasmin ferroxidase activity was measured at 460 nm with o-dianisidine dichloride with intra-and inter-assay coefficients of variation 5.7% and 6.4%, respectively (34). Plasma 17β-estradiol, progesterone, T_3, T_4 and cortisol levels were determined by standard radioimmunoassay techniques using commercial kits (OBRI-ORION, Poland). Intra-and inter-assay coefficients of variation (CV) for 17β-estradiol were 3.8% and 7.9%, for progesterone 4.3% and 5.0%, for T_3 4.2% and 6.8%, for T_4 4.6% and 7.6% and for cortisol 4.8% and 6.9%, respectively. All analyses on plasma obtained from one subject were performed in duplicate and on the same day.

Statistical analysis

Data distribution was evaluated using the Shapiro-Wilk test. Two-ways ANOVA for repeated measures and post-hoc Tukey test were applied for comparison of the variables with normal distribution. Data that were not normally distributed were compared using non-parametric Kruskal-Wallis ANOVA and Mann-Whitney test. Spearman rang correlations between plasma ceruloplasmin ferroxidase activity and TAS levels were also calculated. Statistical significance we set at $p<0.05$. The results were expressed as the mean ± SD. All calculations were performed using STATISTICA v. 7.1 (StatSoft, USA).

Results

There were no differences between ovulating and non-ovulating women with respect to mean values of anthropometric data. However, in non-ovulating women menstrual cycle was significantly longer than in ovulating ones (Table 1).

Both in ovulating and non-ovulating subjects plasma ovarian hormone levels between days 19-22 were markedly higher than between days 5-8 ($p<0.001$) (Table 2). In ovulating participants plasma concentrations of 17β-estradiol were significantly higher than in non-ovulating counterparts both between days 5-8 ($p<0.001$) and between days 19-22 of the menstrual cycle ($p<0.03$). Plasma progesterone levels in ovulating women between days 19-22 of the menstrual cycle were significantly higher ($p<0.001$) than in non-ovulating ones.

Circulating plasma T_3, T_4 and cortisol were not affected by menstrual cycle phase. In addition, in both

Table 1. *Subjects characteristics (mean ± SD)*

	Ovulating women (n=21)	Non-ovulating women (n=16)
Age (years)	20.5 ± 1.2	20.3 ± 1.0
Body mass (kg)	63.3 ± 7.8	62.1 ± 8.2
Body height (cm)	172.4 ± 6.7	169.8 ± 7.0
BMI (kg ·m$^{-2)}$)	21.0 ± 2.1	20.8 ± 2.1
Cycle length (days)	27.8 ± 1.9	30.2 ± 3.5[a]

[a] p<0.01 – significantly different from ovulating women

Table 2. *Plasma hormones, copper and total antioxidant status (TAS) levels and ceruloplasmine ferroxidase activity in ovulating and non-ovulating women (mean ± SD)*

	Ovulating women (n=21)		Non-ovulating women (n=16)	
	Days 5-8	Days 19-22	Days 5-8	Days 19-22
17β-estradiol (pmol · l^{-1})	206.5 ±103.9	525.8 ± 122.3[a]	94.9 ± 22.0[c]	395.5 ± 172.0[a,d]
Progesterone (nmol · l^{-1})	2.2 ± 0.6	38.5 ± 13.1[a]	2.1 ± 0.6	5.1 ± 3.8[b,c]
T_3 (nmol · l^{-1})	0.88 ± 0.14	0.86 ±0.11	0.92 ± 0.48	0.90 ± 0.20
T_4 (nmol · l^{-1})	84.0 ± 10.7	81.4 ± 10.3	83.0 ±12.0	81.7 ± 13.7
Cortisol (µmol · l^{-1})	439.4 ± 106.3	416.6 ± 143.3	458.6 ±182.2	415.5 ± 102.6
Copper (µmol · l^{-1})	14.9 ± 1.8	14.8 ± 1.7	15.4 ± 3.1	15.2 ± 3.2
Ceruloplasmin (U · l^{-1})	53.5 ± 18.8	59.3 ± 16.5	73.9 ± 23.9[e]	75.0 ± 24.3[e]
Ceruloplasmin/Copper*	3.6 ± 1.2	3.9 ± 1.0	4.7 ± 1.0[f]	4.9 ± 0.8[f]
TAS (mmol · l^{-1})	1.3 ± 0.1	1.3 ± 0.15	1.7 ± 0.4[f]	1.7 ± 0.4[f]

* U · l^{-1} to µmol · l^{-1} ratio
[a] p< 0.001, [b] p<0.02 – significantly higher vs. days 5-8; [c] p<0.001, [d] p <0.05 – significantly different vs. respective values in ovulating women;
[e] p<0.01 – significantly higher vs. respective days in non-ovulating women; [f] p<0.02 – significantly higher vs. respective days in ovulating women

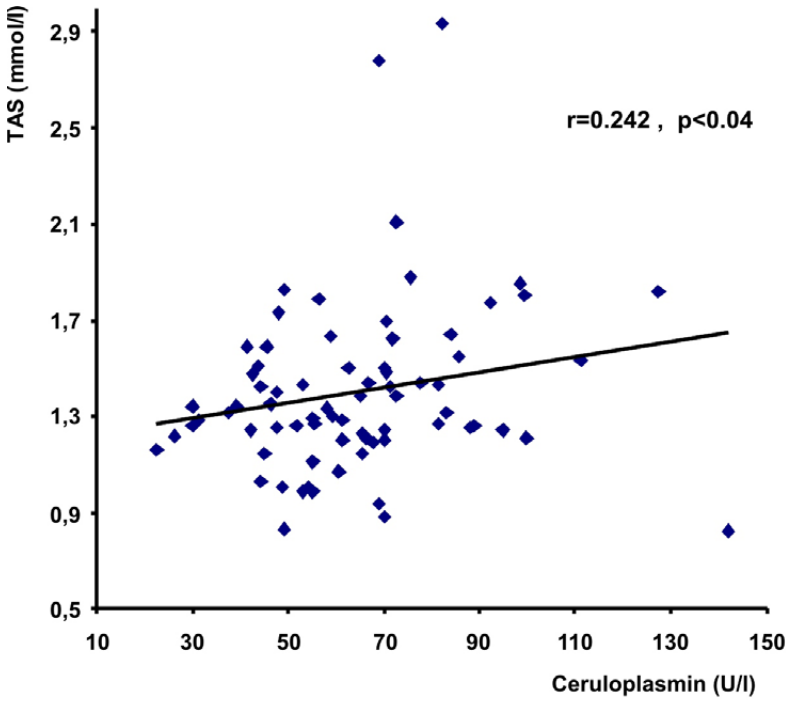

Fig. 1. *The relationship between TAS level and ceruloplasmin ferroxidase activity in collected data from ovulating and non-ovulating women*

cycle phases plasma hormone concentrations were similar in ovulating and non-ovulating women..

Neither in ovulating nor in non-ovulating women plasma copper and TAS levels and ceruloplasmin ferroxidase activity differed in two phases of the menstrual cycle.

However, in non-ovulating women in both cycle phases ceruloplasmin ferroxidase activity and ceruloplasmin ferroxidase activity to copper ratio were higher than in ovulating ones ($p<0.01$ for ceruloplasmin ferroxidase activity and $p<0.02$ for ceruloplasmin ferroxidase activity to copper ratio). Similarly plasma TAS concentrations in non-ovulating women were higher than in their ovulating counterparts ($p<0.02$).

In collected data for ovulating and non-ovulating women plasma TAS levels were slightly but significantly correlated with ceruloplasmin ferroxidase activity ($r=0.242$, $p<0.04$) (Fig. 1). No correlations were noted between circulating hormones and ceruloplasmin ferroxidase activity and between plasma hormones and TAS levels.

Discussion

The most important finding of the current study concerned the stimulatory effect of anovulatory menstrual cycles on plasma TAS levels and ceruloplasmin ferroxidase activity. Furthermore, similar plasma copper levels in non-ovulating and ovulating women suggested that anovulatory menstrual cycles did not affect ceruloplasmin synthesis and secretion from the liver. This assumption is supported by data which indicated that majority of circulating copper (90-95%) is bound to ceruloplasmin (35). Thus, no effect of ovarian hormone secretion on plasma copper levels enable hypothesis to be made that disturbed circulating sex hormones increased ceruloplasmin ferroxidase activity with no effect on plasma ceruloplasmin concentration.

The reason of these findings is unclear. As was mentioned in the introduction ceruloplasmin synthesis in the liver and it plasma levels are stimulated by ovarian hormones (30,31). According to the above data higher ceruloplasmin ferroxidase activity has to be expected in ovulating than in non-ovulating women.

On the other hand, it is worthy to note that ceruloplasmain ferroxidase activity was markedly stimulated by thyroid hormones (36). But neither T_3 nor T_4 plasma levels differed in ovulating and non-ovulating participants of our study.

Significant elevation in plasma ceruloplasmin ferroxidase activity was demonstrated in female rabbits treated with ACTH or cortisol (37). However, in our non-ovulating subjects plasma cortisol levels did not differ in comparison with ovulating counterparts. Thus, elevated ceruloplasmin ferroxidase activity in non-ovulating women were not due to thyroid hormone or cortisol action.

The elevation in plasma ceruloplasmin ferroxidase activity may be induced by oxidative stress observed e.g. in diabetes or atherosclerosis (38,39). On the other side, it has been postulated that the decrease in ceruloplasmin ferroxidase activity reflects increased lipid peroxidation and depressed antioxidant protection (40,41).

The above mentioned discrepancy is mostly due to not fully understood physiological role of ceruloplasmin which acts either as anti- or prooxidant (42).

Taking all the above data together it could be presumed that elevated plasma ceruloplasmin ferroxidase activity in non-ovulating women of the present study indicate either more efficient antioxidant protection or greater oxidative stress.

Similarly, significant elevation in plasma TAS levels noted in non-ovulating women suggested either more efficient antioxidant protection or compensatory response to oxidative stress (43,44).

This assumption seems to be feasible in the context of complicated relationship between two systems - reactive oxygen species production and body antioxidant defense, which are tightly integrated and regulated (45).

It should be noted that in non-ovulating women attenuated estradiol action on erythrocyte dismutase (SOD) activity has been demonstrated accompanied by elevated enzyme activity recognized as adaptational response to increased oxidative stress in the face of depressed ovary function (46).

In the current study no correlation between circulating 17β-estradiol and/or progesterone and plasma TAS as well as between ovarian hormones and ceruloplasmin ferroxidase activity was noted. On the other hand, both plasma TAS and ceruloplasmin feroxidase activity were higher in females with depressed ovary function. Thus, it could be tentatively postulated that low circulating 17β-estradiol and progesterone in non-ovulating women caused oxidative stress which in turn provoked compensatory increase in plasma ceruloplasmin ferroxidase which contributed to elevated total plasma antioxidant potential expressed as plasma TAS levels.

Additionally, week but significant correlation between plasma TAS and ceruloplasmin ferroxidase activity possibly indicated that at least in females they act in concert to improve plasma antioxidant protection. These findings may be of importance especially in physically active women who are at high risk of ovary dysfunction (47).

However, to validate this assumption more data concerning lipid peroxidation and other endogenous plasma antioxidants (e.g. bilirubin, uric acid, thiols) in ovulating and non-ovulating women are needed.

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HEART RATE AND SALIVARY CORTISOL RESPONSES IN ARMCHAIR FOOTBALL SUPPORTERS

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Abstract

Introduction: Sport spectators may experience considerable emotional stress when watching televised events, and this may provoke adverse cardiovascular events.

Aim of the study: We examined cardiovascular and stress responses of English and Swedish supporters watching a televised event involving the two teams at the 2006 FIFA World Cup.

Results: All supporters showed substantial elevations of heart rate when their team scored a goal (the result was 2-2), but supporters showed no response when their team conceded a goal. During the first half, the average heart rate of the Swedes (81±12 b/min) was lower ($p<0.001$) than that of the English supporters (92±11 b/min). Mean heart rate of both sets of supporters was higher in the second half. Heart rate of England supporters peaked when England scored after 34 min (116±14 b/min) and at 86 min (120±12 b/min), with no change in heart rate of the Swedish supporters at these times. Heart rates of Sweden supporters peaked when their team scored after 51 min (107±18 b/min) and 91 min (133±19 b/min). Swedish supporters reported more elation after the game than English supporters and generally higher subjective ratings of anxiety, stress and excitement, but there were no differences in salivary cortisol and amylase data.

Conclusion: Heart rate of spectators responds markedly to events in a televised football game but depends on the subjective perception of events. Salivary cortisol and amylase variables are relatively insensitive measures of psychosomatic stress.

***Key words**: football, soccer, stress, cortisol, heart rate*

Introduction

People watch sporting contests for many different reasons. For some, the role of spectator is an opportunity to escape the monotony of daily life, while for others it may be seen as a relief from the tensions and an escape from the stresses of daily life. Being a spectator, however, can induce significant physiological stress responses, even if watching only on television. Rost et al showed substantial increases in heart rate and arterial blood pressure in spectators watching a televised football World Cup game in 1974 (1).

At its most extreme, a major sporting event may increase stress to such an extent that it precipitates acute myocardial infarction or stroke among some of those spectating. This was shown in a longitudinal study of mortality in The Netherlands around 22 June 1996 (the day the Dutch football team was eliminated from the European football championship) (2). Mortality on 22 June was compared with the five days before and after the match and in the same period in 1995 and 1997. Mortality from coronary heart disease and stroke was increased in men on the day of the match (relative risk 1.51, 95% confidence interval 1.08 to 2.09), but no clear rise in mortality was observed for women during the same period (1.11, 0.80 to 1.56). Among men, about 14 excess cardiovascular deaths occurred on the day of the match. Carroll et al also showed a large (25%) increase in hospital admissions in England for myocardial infarction on the day of and for the two days after England's elimination by Argentina (in a penalty shoot-out) in the quarter-final of the 1998 World Cup (3). In the North of England, a significant increase in the number of deaths attributable to acute myocardial infarction and stroke has been reported to occur in men, but not in women, on days when the local professional football team lost at home (4).

In contrast to these observations, Toubiana et al (5) did not find any change in French cardiovascular mortality during the 1996 European football championships, and Berthier and Boulay (6) showed a reduction in mortality from myocardial infarction, but not in all cause mortality, in French men on the day of the 1998 World Cup final, which was won by France. They also reported a "non-significant decrease" in deaths from myocardial infarction in women on the same day.

These findings suggest that high profile sporting events may have profound medical consequences in some parts of the population and that there may be gender, and possible also ethnic, differences in the response. With the exception of the study of Rost et al (1), however, these data all derive from retrospective studies of hospital admissions records, and therefore do not distinguish between events occurring during a game and those that take place in the aftermath of the game. They also take no account of the extent of the physiological responses that occur during the events to which they relate. The aim of this study was to measure some markers of the stress response in individuals

watching a live television broadcast of a game at the 2006 Football World Cup competition

Methods

Subjects were 20 healthy adults, 10 of whom (mean±SD age 32±15 y, Body Mass Index 24.1±3.7 kg·m^{-2}) were English Nationals supporting England (all male) and 10 of whom (6 m, 4 f; age 26±4 y, Body Mass Index 22.1±2.1 kg·m^{-2}) were Swedish nationals who were supporting Sweden. All were members of supporters' clubs and were committed to supporting their team. Subjects were recruited by personal contact and were invited to watch the England-Sweden game, the last game in the qualifying round of the 2006 World Cup football competition on 20 June 2006. The England team had qualified to progress to the next stage of the competition, regardless of the outcome, but a loss would mean elimination for the Swedish team. The purpose of the study, which was approved by the institutional research ethics committee, was explained to participants before they signed a consent form. Subjects watched the game on a wide screen in a relaxed environment: all were seated comfortably in the same type of chair, but were not required to remain seated throughout.

Heart rate was recorded continuously throughout the game (Polar Team System, Polar Electro, Kempele, Finland). Data were downloaded to a computer after the game and recorded as successive 1-minute average values. Non-stimulated saliva samples were collected over a timed 3-minute period before the game, at the beginning of the half-time interval and again immediately upon conclusion of the game. Food and drink were available to participants except for the last 15 min before sample collections. Samples were stored on ice and analyzed for cortisol by ELISA (DRG Instruments, GmbH, Germany) and α-amylase activity using a spectrophotometric kinetic assay (Salimetrics, Philadelphia, USA). Saliva flow rate was estimated from the weight of saliva collected assuming the density of saliva to be 1.0 g/ml. Subjective arousal was assessed at the beginning of the half-time interval and again immediately upon conclusion of the game using a 10 cm visual analogue scale with three questions relating to the supporters' feelings of anxiety, stress and excitement. The extremes of the scales were "Not at all" and "Extremely". A fourth question relating to supporters' feelings of elation was added after the game.

Data are expressed as Mean±SD. Comparisons were made using the Student's t-test for paired or unpaired comparisons as appropriate.

Results

No differences between the male and females Swedish participants were noted, so data have been pooled. There was no difference in the heart rate response of male (85±10 b/min) and female (86±19 b/min) Swedish supporters (p=0.108). Average heart rate data for the two sets of supporters over the whole duration of the game are presented in Figure 1. During the first half of the game, the average heart rate of the Swedes

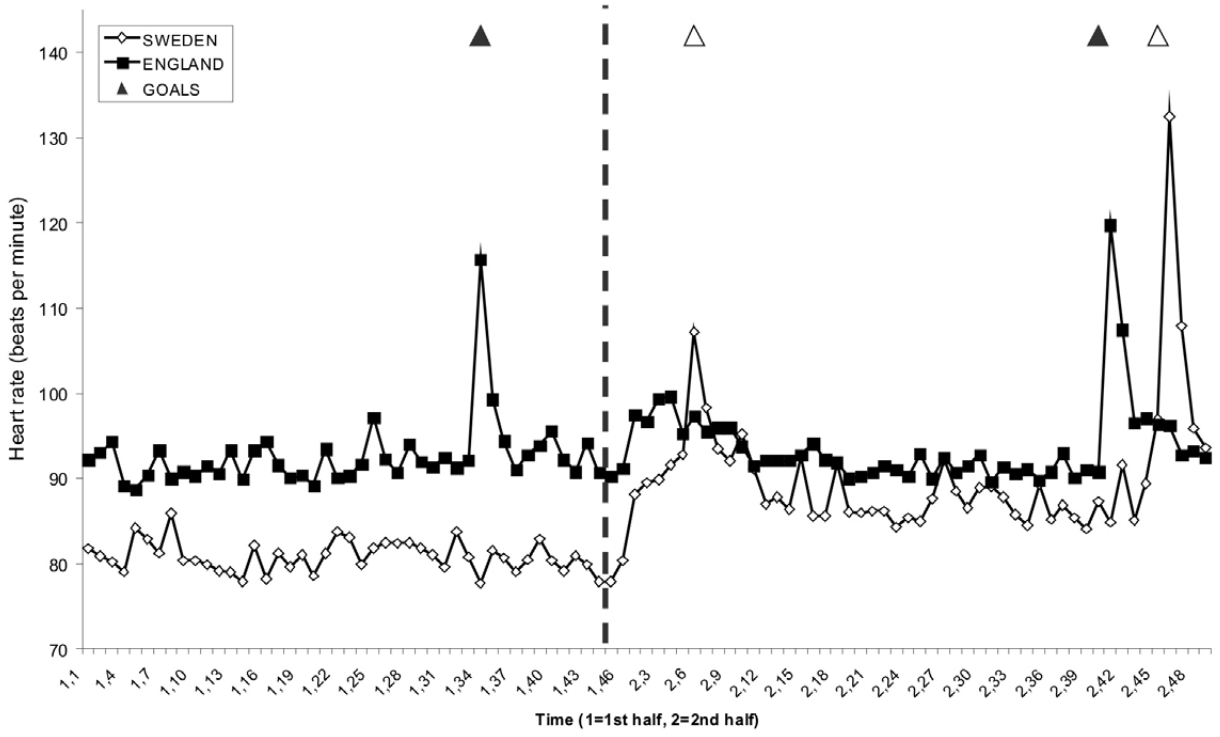

Fig. 1. *Mean Heart rate data for England and Sweden supporters throughout the* game. *England goals are shown in solid triangles and Sweden goals in open triangles.*

Table 1. *Self-reported levels of anxiety, stress and excitement. Values are Mean ± SD, n = 10 in each group, p values indicate significant differences between Swedish and English supporters.*

Anxiety			
	Pre-match	Half-time	Full-time
England	2.7 ± 2.0	3.4 ± 1.9	6.3 ± 2.0
Sweden	3.9 ± 2.3	6.1 ± 2.4	6.1 ± 3.6
		p<0.05	
Stressed			
	Pre-match	Half-time	Full-time
England	2.8 ± 1.8	3.6 ± 2.2	6.1 ± 1.4
Sweden	4.1 ± 2.7	5.9 ± 2.5	6.8 ± 3.5
		p<0.05	
Excitement			
	Pre-match	Half-time	Full-time
England	4.9 ± 1.3	5.4 ± 1.3	4.8 ± 3.2
Sweden	7.3 ± 2.0	5.8 ± 2.8	8.2 ± 1.5
	p<0.01		p<0.01

(81±12 b/min) was lower (p<0.001) than that of the English supporters (92±11 b/min). Mean heart rate of the Swedes was higher in the second half than in the first (90±16 b/min; p<0.001), and a similar pattern was seen for the England supporters (94±9 b/min; p<0.005). The heart rate of the Swedish supporters in the second half was still lower than that of the English supporters (p<0.001). Heart rate of the England supporters peaked when England scored after 34 min (116±14 b/min) and again at 41 min into the second half (120±12 b/min): there was no change in heart rate of the Swedish supporters at these times. Heart rates of the Sweden supporters peaked twice during the second half, when the Swedish team scored after 6 min (107±18 b/min) and 46 min (133±19 b/min): there was no change in the heart rate of the England supporters at these times. A substantial variation between individuals in the heart rate response was noted. No record of movement during the match was made, so the possible relationship with movement cannot be explored. Observation of the subjects, however, suggests that this does not account for the individual differences.

In spite of the trend for a lower heart rates among the Swedes, their self-reported levels of anxiety, stress and excitement were generally higher than those of the English as illustrated in Table 1. After the game, the feeling of elation was greater among the Swedes (6.1±2.8) than among the English supporters (1.8±1.2; p<0.025).

The saliva data revealed a tendency for the average saliva cortisol concentration to increase in the Swedish supporters over the course of the game, with relatively little change in the English (Table 2), though there were no statistically significant differences between the two groups of supporters. The saliva cortisol secretion rates were also not different between the two groups, but the values for the Swedes were significantly higher at half-time compared with their pre-match values (p<0.03). The average saliva α-amylase secretion rate (U·min^{-1}) was 30-40% higher in both sets of supporters (Table 2) at both half time (58±46 U·min^{-1}, p<0.04) and full time (60±43 U·min^{-1}, p<0.04) compared with before the match (43±32 U·min^{-1}), reflecting the higher levels of perceived stress (Table 1) in both groups of supporters while watching the match compared with beforehand. However, there were no significant differences in the salivary amylase activity or secretion rate between the two groups of supporters.

Table 2. *Salivary flow rate, cortisol concentration, cortisol secretion rate and amylase secretion rate. Values are Mean ± SD, n = 10 in each group. There were no significant differences between the two groups of supporters, but the saliva cortisol secretion rate was significantly elevated at half-time in the Swedes compared with their pre-match value (*p<0.05). In both groups of supporters, the saliva amylase secretion rate was elevated at half-time and full-time compared with pre-match values (*p<0.05)*

Saliva Flow rate (ul·min^{-1})			
	Pre-match	Half-time	Full-time
England	343 ± 136	435 ± 191	420 ± 182
Sweden	537 ± 383	685 ± 461	506 ± 298
Saliva Cortisol concentration (ng·ml^{-1})			
	Pre-match	Half-time	Full-time
England	3.4 ± 1.1	2.9 ± 0.5	2.8 ± 0.4
Sweden	2.5 ± 1.0	2.8 ± 1.1	3.1 ± 1.0
Saliva Cortisol secretion rate (ng·min^{-1})			
	Pre-match	Half-time	Full-time
England	1.1 ± 0.4	1.2 ± 0.5	1.1 ± 0.4
Sweden	1.3 ± 1.0	1.6 ± 0.9*	1.4 ± 0.6
Saliva Amylase secretion rate (U·min^{-1})			
	Pre-match	Half-time	Full-time
England	36 ± 30	46 ± 37*	50.8 ± 38*
Sweden	50 ± 35	69 ± 52*	69.8 ± 47*

Discussion

Although the measures made in the present study are rather simplistic, it is clear that the physiological and subjective responses of spectators to events taking place in televised competitions a very strongly influenced by the perception of that event. Heart rates of supporters rose markedly when their team scored a goal, but there was no response when their team conceded a goal.

It is well recognized that the response to events such as viewing sport or filmed violence is strongly influenced by the environment and by the behavior of others (7). In spite of this, however, the variation between individuals was large, though the reasons for this inter-individual variability in response are not at all clear. Among the 20 subjects the highest average heart rate recorded during the whole match for an individual was 115 b/min and the lowest was 74 b/min. Even though the subject numbers are small, the heart rate and other data suggest that the response of male and female supporters watching a televised game is not different. This is in contrast to previous reports which suggest that male spectators show a greater degree of involvement than females (8,9).

A previous report of reactions of spectators to watching a televised World Cup game concluded that there were substantial elevations of heart rate and blood pressure in spectators, but the numbers were small (n = 5) and the measurements made were limited (1). The striking feature of the subjects in the present study, as illustrated in Figure 1, is the dramatic elevation of heart rate when "their" team scores a goal and the complete absence of any response when the opposing team scored. The highest individual heart rate achieved, averaged over a full minute was 168 b/min. In part, the high heart rates recorded after goals scored are probably attributable to an increase in the level of physical activity (standing up, jumping, clapping, arm waving) as the supporters celebrate "their" team scoring. Thus, the average heart rate over the whole match is perhaps a better indicator of the overall level of arousal and psychosomatic stress experienced by individuals.

The Swedish supporters in the present study generally had a lower heart rate than the England supporters, in spite of a higher self-reported level of anxiety, stress and excitement. This may reflect a greater level of aerobic fitness in the Swedes, as a low resting heart rate, consequent upon a greater stroke volume, has long been recognized to be a characteristic of aerobic fitness (10). Alternatively, it may reflect a smaller degree of sympathetic stimulation, as the heart rates generally are higher than would be expected in resting young adults. The higher heart rates of the Swedes during the second half is consistent with an increased level of arousal due to the greater pressure on their team at this time. The much smaller increase seen in the English supporters may reflect the relative lack of importance of the match outcome to their team's progression in the tournament.

There are suggestions in the literature to support the idea that there may be cultural differences in reactions to watching live or televised sporting events. There is strong evidence for changes in the pattern of cardiovascular events in some populations in the period during and immediately after a major televised football event in the Netherlands (2), the UK (3) and in Switzerland (11, 12). Others, however, have found no effect when similar surveys have been carried out in France (6) and in Australia (13). From the US, there is evidence of an increased incidence of domestic violence when the local home (American) football team wins (14).

The saliva α-amylase secretion rate is reported to reflect endogenous adrenergic activity, but this was not different between the two groups of supporters at any time (15). It did raise during the first half of the game, however, and rose further still by the end of the game. The subjective ratings of anxiety, stress and excitement were generally higher among the Swedish supporters, but there was no difference in the measures salivary variables. Although the subject number in the present study is small, the absence of differences in salivary cortisol and amylase data between the two groups of supporters, suggests that these salivary variables may be relatively insensitive measures of psychosomatic stress.

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