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The effects of acute and chronic Red Bull® consumption on cardiodynamics and oxidative stress in coronary effluent of trained rats

Efekti akutne i hronične konzumacije Red Bull®-a na kardiodinamiku i oksidativni stres u koronarnom efluentu treniranih pacova

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Abstract

Background/Aim. Energy drinks (EDs) are widely used by athletes as ergogenic agents and Red Bull® is one of the most consumed EDs among them. The aim of this study was to determine the acute and chronic effects of Red Bull® on cardiodynamics and parameters of oxidative stress in physically trained rats. **Methods.** Rats were subjected to a swimming practice (1h a day, 5 days a week, for 4 weeks). They were divided into 4 groups: rats that did not consume ED either before swimming or prior to sacrificing; rats that did not consume ED before swimming but did consume ED 30 min prior to sacrificing; rats that consumed ED 30 min before every swimming training but did not consume ED prior to sacrificing; rats that consumed ED 30 min before every swimming training and 30 min before sacrificing. After sacrificing, the hearts of the rats were isolated and perfused according to the Langendorff technique. The parameters of cardiac function were recorded, and also the levels of prooxidants were measured in the coronary effluent during coronary autoregulation. **Results.** Acute administration of the ED had a positive inotropic effect (manifested as a significantly higher level of the maximum and minimum rate of pressure development in the left ventricle), while chronic administration affected the isolated increase in systolic left ventricular pressure. The prooxidative effect of the ED was observed, which was more pronounced in chronic consumption. **Conclusion.** The main conclusion of our study is that chronic consumption of ED changes the cardiovascular response and redox status in acute consumption ED.

Key words:
energy drinks; exercise; cardiovascular system; oxidative stress; rats; swimming.

Apstrakt

Uvod/Cilj. Energetska pića (EP) se često koriste kao ergogena sredstva od strane sportista, a Red Bull® je jedno od najčešće konzumiranih EP. Cilj rada bio je da se utvrde akutni i hronični efekti Red Bull®-a na kardiodinamiku i parametre oksidativnog stresa kod fizički treniranih pacova. **Metode.** Pacovi su bili podvrgnuti plivanju (1 h dnevno, 5 dana u nedelji, tokom 4 nedelje) i podeljeni u 4 grupe: pacovi koji nisu konzumirali EP ni pre plivanja, ni pre žrtvovanja; pacovi koji nisu konzumirali EP pre plivanja, ali jesu 30 min pre žrtvovanja; pacovi koji su konzumirali EP 30 min pre svakog plivanja, ali ga nisu konzumirali pre žrtvovanja; pacovi koji su konzumirali EP 30 min pre svakog plivanja i 30 min pre žrtvovanja. Nakon žrtvovanja, srca pacova su bila izolovana i perfundovana prema tehnici po Langendorff-u. Određivani su parametri funkcije srca, kao i nivo prooksidantnih vrsta u koronarnom efluentu tokom koronarne autoregulacije. **Rezultati.** U poređenju sa kontrolnom grupom, akutna primena EP imala je pozitivan inotropni efekat, značajno povećanje maksimalne i minimalne stope promene pritiska u levoj komori, dok je hronična konzumacija uticala na izolovano povećanje sistolnog arterijskog pritiska. Zapaženi su prooksidativni efekti EP, što je bilo izraženije kod hronične konzumacije EP. **Zaključak.** Glavni zaključak ove studije jeste da hronična konzumacija EP menja kardiovaskularni odgovor i redoks status prilikom akutne primene EP.

Ključne reči:
energetski napici; vežbanje; kardiovaskularni sistem; stres, oksidativni; pacovi; plivanje.

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Introduction

Energy drinks (EDs) are beverages with stimulating effects due to a combination of specific ingredients [1, 2]. The main active ingredient of these drinks is caffeine but it has been shown that other components also contribute to the changes in the work of the cardiovascular system [3–7]. EDs are consumed to provide additional energy, increasing cognitive and physical performance, prolonging alertness, and improving mood [8]. Due to the positive inotropic effect, they should induce some benefit to exercising individuals by improving skeletal muscle oxygenation and increasing aerobic metabolism and muscular performance [9]. Thus, EDs are widely used by athletes as ergogenic agents [10].

Red Bull® (RB®) is considered to be one of the most consumed EDs [4, 11]. It has been shown that 355 mL of RB® leads to a significant increase in the systolic and diastolic blood pressure, heart rate, stroke volume, and a double product, which is an indirect indicator of oxygen consumption in the myocardium [12]. Because of the significant increase in myocardial function of the right and left ventricles (LVs), a positive inotropic effect of EDs has been suggested [13]. EDs affect the increase in glycemia, cholesterol, and triglycerides [14]. Thus the overall effect of EDs represents an increase in cardiovascular risk [15]. Acute cardiovascular adverse effects of EDs include the effect on hemodynamics and electrophysiological changes, the effect on endothelial function, and the association with vascular pathology [16]. The association between ED consumption and cardiovascular changes includes supraventricular and ventricular arrhythmias, ischemia and myocardial infarction, QT interval prolongation, aortic dissection, and death [7]. Although moderate consumption of EDs is considered relatively safe in healthy population [10], their consumption is not recommended in sports and during exercise, and special caution is recommended for people with cardiovascular diseases [17, 18].

The occurrence of oxidative stress in a blood vessel and damage to the endothelium-dependent vasodilation is associated with a reduction in the production of nitric oxide (NO) or the increased production of the reactive oxygen species (ROS), in particular of superoxide anion (O_2^-) [19]. The effect of caffeine on blood vessel regulation is manifested through the balance of vasoconstrictor and vasodilatory effect [20]. At rest, caffeine either improves or does no alteration [21] to the endothelial function [20]. However, during physical activity, this function is reduced [21]. Current data suggest that EDs decrease endothelial function at rest [22, 23]. In mice, it is shown that not only does the application of EDs affect the reduction of peri-intestinal fat tissue, but it also increases the pericardial fat tissue, which represents a significantly greater source of chemokines and cytokines with proinflammatory properties, compared to the subcutaneous fat tissue [24], which further implies a larger production of ROS [25]. Particular importance is attributed to oxidative stress because it plays an important role in pathogenesis and the development of cardiovascular diseases.

There are very few preclinical studies in the literature examining the influence of EDs on the cardiovascular system. The aim of this study was to determine the acute and chronic effects of RB® on cardiodynamics and oxidative stress in the coronary effluent in physically trained rats. The study also aimed to determine the effect of chronic RB® consumption on the changing of the indicated parameters (cardiodynamics and oxidative stress) in the presence of an acute RB® consumption.

Methods

The study was conducted in the Laboratory for Cardiovascular Physiology at the Faculty of Medical Sciences, University of Kragujevac. It was approved by the Ethics Committee of the Faculty. Good Laboratory Practice and the European Council Directive (86/609EEC) were followed during the conception, design and performance of the study.

Subjects

The study was performed using the Wistar albino rats. The sample size calculation, based on a study published by Barcelos et al. [26], revealed that 24 rats were requisited to perform the study. At the beginning of the study, the rats were eight weeks old and weighed 200–250 g. They were kept in cages (8 rats in one cage), fed with commercial rat food (20% protein food, Veterinary Institute Subotica, Serbia), and watered ad libitum. The temperature in the room was set to 25°C, and 12 hours of light were provided.

Training protocol

The study lasted for 4 weeks. All rats were subjected to a swimming practice (1h a day, 5 days a week) in an 80 × 60 × 100 cm pool for experimental animals. An electric heater was used to keep the water temperature at 34°C. During swimming, the pump installed in the pool made constant waves, in order to prevent the rats from floating. Rats were constantly monitored during swimming.

ED consumption

Initially, rats were divided into two groups based on ED consumption during the study period (rats that did and did not consume ED 30 min before swimming). Later those two groups were further divided into groups based on ED consumption before the sacrificing (rats that did and did not consume ED before they were sacrificed). Thus, groups were formed as follows: control group (C-T) – rats that did not consume ED either before swimming or prior to sacrificing (n = 6); acute ED group (acED-T) – rats that did not consume ED before swimming, but did consume ED 30 min prior to sacrificing (n = 6); chronic ED group (chED-T) – rats that consumed ED 30 min before every swimming training, but did not consume ED prior to sacrificing (n = 6); chronic + acute group (ch + acED-T) – rats that consumed

ED 30 min before every swimming training and 30 min before sacrificing (n = 6).

The ED administration was performed by an intragastric gavage (p.o.). RB® was used in the amount of 3.75 mL/kg, as determined on the basis of the previously published studies [26, 27]. The indicated dose corresponds to a dose of caffeine close to the maximum recommended (about 6 mg/kg). A standard of 250 mL RB® contains the following: 80 mg of caffeine, 1,000 mg of taurine, 21.5 g of sucrose, 5.25 g of glucose, 600 mg of glucuronolactone, 20 mg of vitamin B3 (niacinamide), 5 mg of vitamin B5 (calcium pantothenate), 5 mg of vitamin B6 (pyridoxine hydrochloride), 50 mg of inositol, 5 μg of vitamin B12 (cyanocobalamin), 100 mg of sodium citrate, as well as natural and artificial flavors and colors (caramel, riboflavin) [6, 28, 29].

Cardiodynamic parameters

After short ketamine/xylazine narcosis, rats were sacrificed, and their hearts were excised and attached to the Langendorff apparatus via aortic cannula. Krebs-Henseleit buffer was used during the performance of the retrograde perfusion according to the Langendorff technique. The equilibration period, during which coronary perfusion pressure (CPP) was kept at 70 cm H_2O, was performed first. After that, CPP was changed in the following order: 1) 60 cm H_2O, 2) 80 cm H_2O, 3) 100 cm H_2O, 4) 120 cm H_2O, and 5) 40 cm H_2O.

Parameters of myocardial function were measured using the pressure sensor (transducer BS4 73-0184, Experimentria Ltd, Hungary) attached to the latex balloon, filled with bubble-free saline, which was inserted into the left chamber [30]. Cardiodynamic parameters were continuously measured. The following parameters of myocardial function were recorded: 1) the maximum and minimum rate of pressure development in LV (dP/dt max and dP/dt min), 2) systolic LV pressure (SLVP) and diastolic LV pressure (DLVP), and 3) heart rate (HR). Furthermore, coronary flow (CF) was measured flowmetrically.

Oxidative stress

CF collected during each CPP was used to measure the levels of oxidative stress in coronary venous effluent. A spectrophotometer (Analytic Jena Specord S 600, UK) was used to determine the levels of the following: 1) superoxide anion radical (O_2^-), 2) hydrogen peroxide (H_2O_2), 3) nitrogen monoxide (NO), and 4) index of lipid peroxidation (TBARS). The exact protocols for measurement of those prooxidative species may be found in our previously published papers [31] or in the original sources [32–35].

Statistics

SPSS 23.0 was used to perform the statistical analysis. Comparison of groups was performed using the parametric (*t*-test for independent samples) or nonparametric tests (Mann-Whitney *U* test), depending on the results of the Shapiro-Wilk test for data distribution. The results in the figures are shown as the mean ± standard error (SE) of the mean.

Results

Cardiodynamics

Cardiodynamic parameters of isolated rat hearts in four groups (C-T, acED-T, chED-T, ch + acED-T) are shown in Figures 1–6.

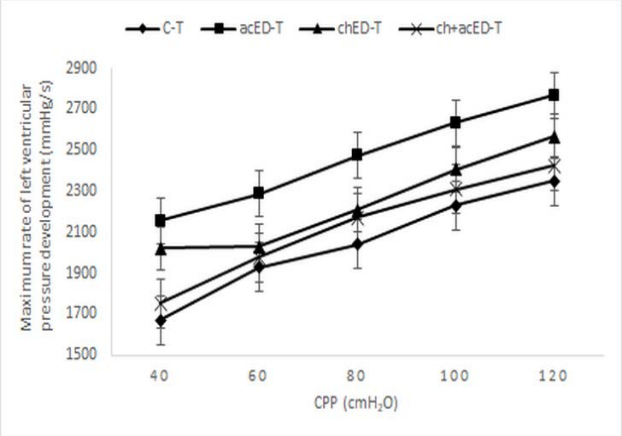

Fig. 1 − Values of the maximum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T (data are given as means ± standard error).
C-T – control group; acED-T – acute energy drinks (ED-T) group; chED-T – chronic ED-T group; ch + acED-T – chronic + acute ED-T groups; CPP – coronary perfusion pressure.

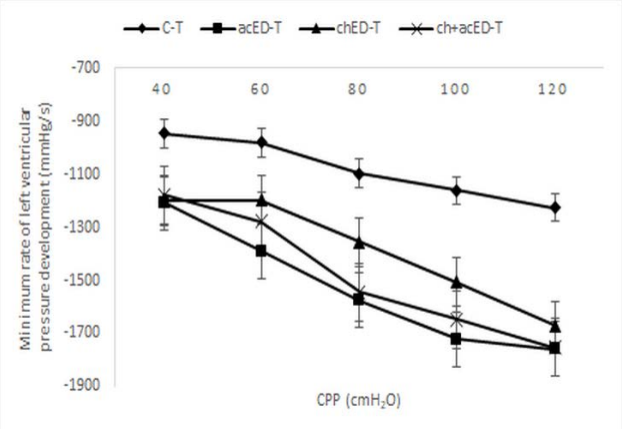

Fig. 2 − Values of the minimum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

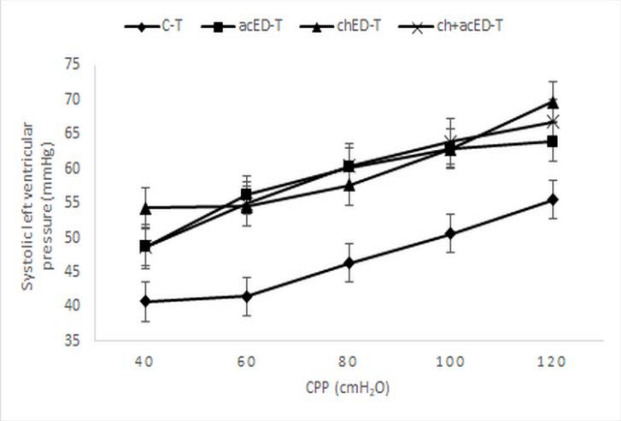

Fig. 3 − Values of systolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

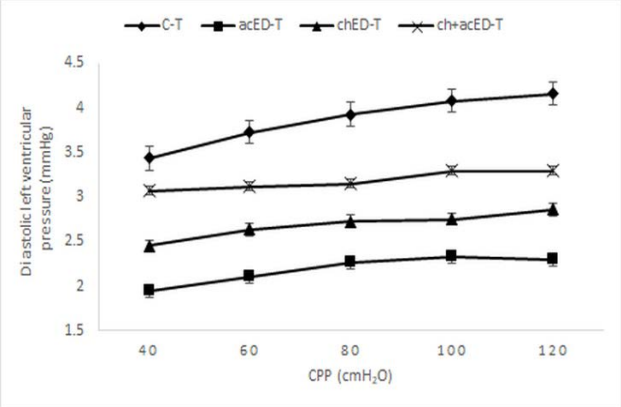

Fig. 4 − Values of diastolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

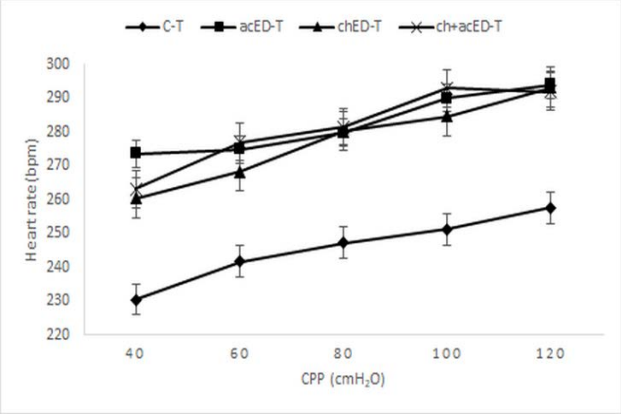

Fig. 5 − Values of heart rate during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

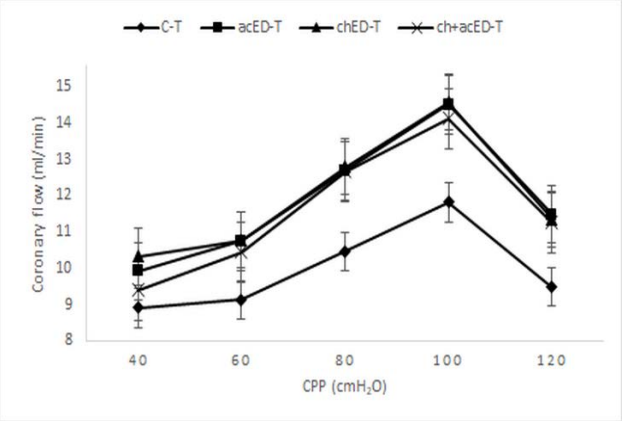

Fig. 6 − Values of coronary flow during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

Concerning the C-T group, the following results were recorded in the acED-T group: 1) at all CPPs, statistically significantly higher level of dP/dt max ($p < 0.05$; *t*-test for independent samples), significantly higher level of HR ($p < 0.05$; Mann-Whitney test) and a significantly lower level of DLVP ($p < 0.05$; *t*-test for independent samples); 2) at CPP 60–100 cm H_2O, statistically significantly higher level of SLVP ($p < 0.05$; *t*-test for independent samples); 3) at CPP 60–120 cm H_2O, statistically significantly higher levels of dP/dt min and CF ($p < 0.05$; *t*-test for independent samples).

Concerning the C-T group, the following results were recorded in the chED-T group: 1) at all CPPs, statistically significantly higher level of HR ($p < 0.05$; *t*-test for independent samples) and significantly lower level of DLVP ($p < 0.05$ Mann-Whitney test); 2) at CPP 60–120 cm H_2O, statistically significantly higher level of CF ($p < 0.05$; Mann-Whitney test); 3) at all CPP, higher level of SLVP, but only statistically significantly higher at CPP 60, 100 and 120 cm H_2O ($p < 0.05$; *t*-test for independent samples) and higher level of dP/dt min, but statistically significant only at CPP 100–120 cm H_2O ($p < 0.05$; *t*-test for independent samples); 4) at all CPP, higher level of dP/dt max, but without statistical significance ($p > 0.05$; *t*-test for independent samples).

Concerning the acED-T group, the following results were recorded in the ch + acED-T group: 1) at all CPPs, lower level of dP/dt max and higher level of DLVP, but statistically significant for both parameters only CPP 40 cm H_2O ($p < 0.05$; *t*-test for independent samples); 2) at all CPP, lower level of dP/dt min and CF, but without statistical significance ($p > 0.05$; *t*-test for independent samples). There were no statistically significant differences in the levels of SLVP and HR between these two groups ($p > 0.05$; Mann-Whitney test).

Concerning the chED-T group, the ch + acED-T group recorded a lower level of dP/dt max and CF, as well as a higher level of DLVP, but without statistical significance (at all CPPs) ($p > 0.05$; *t*-test for independent samples; Mann-

Whitney test). There were no statistically significant differences in the levels of SLVP, dP/dt min, and HR between these two groups ($p > 0.05$; Mann-Whitney test; *t*-test for independent samples).

Concerning the C-T group, the following results were recorded in the ch+acED-T group: 1) at all CPPs, a significantly higher level of HR ($p < 0.05$; *t*-test for independent samples), higher level of SLVP, but only at CPP 60 and 80 cm H_2O statistically significantly higher ($p < 0.05$; Mann-Whitney test) and higher level of dP/dt min, but at CPP 60–120 cm H_2O statistically significantly higher ($p < 0.05$; *t*-test for independent samples); 2) at all CPPs, higher level of dP/dt max and CF, and lower level of DLVP, but without statistical significance ($p > 0.05$; *t*-test for independent samples).

Oxidative stress

Prooxidative parameters in the effluent during coronary autoregulation of isolated rat hearts in four groups (C-T, acED-T, chED-T, ch + acED-T) are shown in Figures 7–10.

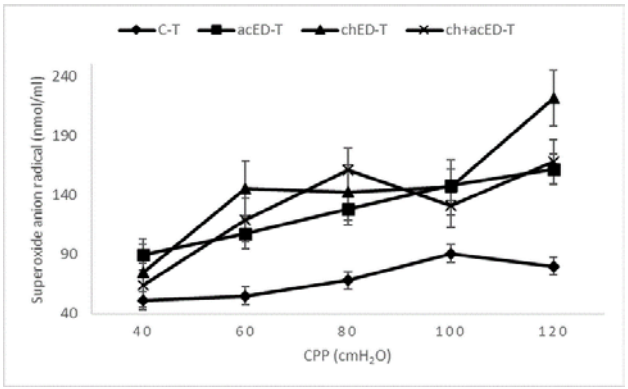

Fig. 7 − Values of superoxide anion radical in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T (data are given as means ± standard error). For abbreviations see under Figure 1.

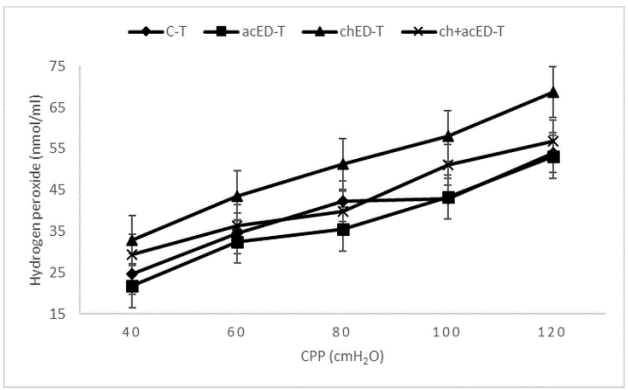

Fig. 8 − Values of hydrogen peroxide in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error). For abbreviations see under Figure 1.

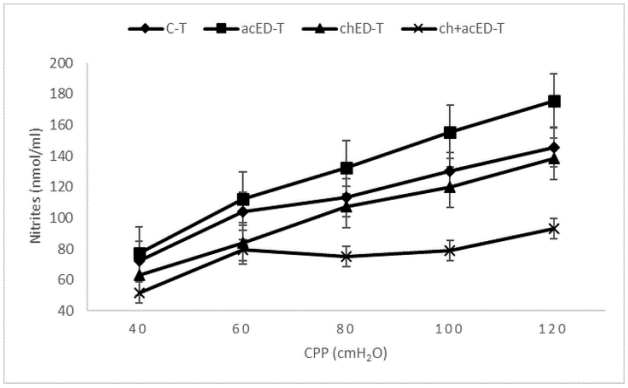

Fig. 9 − Values of nitrites in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T (data are given as means ± standard error). For abbreviations see under Figure 1.

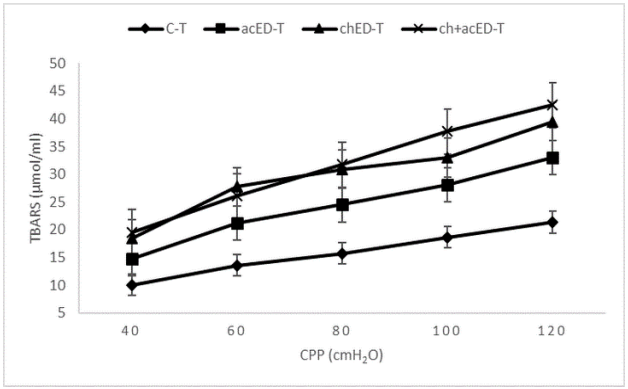

Fig. 10 − Values of index of lipid peroxidation (TBARS − thiobarbituric acid reactive substances) in effluent during coronary autoregulation of the isolated trained rat heart in the following groups: C-T, acED-T, chED-T, ch + acED-T (data are given as means ± standard error). For abbreviations see under Figure 1.

In relation to the C-T group, the following results were recorded in the acED-T group: 1) at all CPP, the level of TBARS (lipid peroxidation index) was statistically significantly higher ($p < 0.05$; Mann-Whitney test); 2) at all CPP, levels of O_2^- and nitrites (NO) were higher, but without statistical significance ($p > 0.05$; Mann-Whitney test; $p > 0.05$; *t*-test for independent samples, respectively). There was no statistically significant difference in the level of H_2O_2 between these two groups ($p > 0.05$; Mann-Whitney test).

In relation to the C-T group, the following results were recorded in the chED-T group: 1) at all CPP, the level of TBARS was statistically significantly higher ($p < 0.05$; Mann-Whitney test); 2) at CPP 60, 80, and 120 cm H_2O, the level of O_2^- was statistically significantly higher ($p < 0.05$; Mann-Whitney test); 3) at all CPP, level of H_2O_2 was higher but statistically significant only at CPP 60 cm H_2O ($p < 0.05$; Mann-Whitney test); 4) at all CPP, levels of nitrites (NO) were lower, but without statistical significance ($p > 0.05$; Mann-Whitney).

In relation to the acED-T group, the following results were recorded in the ch + acED-T group: 1) at all CPPs, the

level of TBARS was statistically significantly higher ($p < 0.05$; *t*-test for unbound samples); 2) at CPP 40, 80, 100 and 120 cm H_2O, the levels of NO were statistically significantly lower ($p < 0.05$; Mann-Whitney test); 3) at all CPP, level of H_2O_2 was higher, but without statistical significance ($p > 0.05$; Mann-Whitney test). There was no statistically significant difference in the level of O_2^- between these two groups ($p > 0.05$; Mann-Whitney test).

In relation to the chED-T group, the following results were recorded in the ch + acED-T group: 1) at all CPPs, the levels of NO were lower, but statistically significant only at CPP 80–120 cm H_2O ($p < 0.05$; Mann-Whitney test); 2) at all CPPs, the level of H_2O_2 was lower, but without statistical significance ($p > 0.05$; Mann-Whitney test). There were no statistically significant differences in the levels of O_2^- and TBARS between these two groups (p > 0.05; Mann-Whitney test; $p > 0.05$; *t*-test for independent samples).

In relation to the C-T group, the following results were recorded in the ch + acED-T group: 1) at all CPPs, the level of TBARS was statistically significantly higher ($p < 0.05$; Mann-Whitney test), while the level of O_2^- was higher, but statistically significant only at CPP 60 and 80 cm H_2O ($p < 0.05$; Mann-Whitney test); 2) at all CPPs, the levels of nitrites (NO) were lower, but statistically significant at CPP 40, 80–120 cm H_2O ($p < 0.05$; Mann-Whitney test). There was no statistically significant difference in the level of H_2O_2 between these two groups ($p > 0.05$; *t*-test for independent samples).

Discussion

In this research, we studied the effect of RB® on cardiovascular parameters and coronary autoregulation in the isolated heart of trained rats, as well as the level of prooxidative parameters in the coronary venous effluent. The training included swimming for four weeks (1 hour/day, 5 days/week), which is considered to be a moderate-intensity exercise. The specificity of swimming as an exercise is in engaging the muscles of the entire body, which improves the capacity of the cardiovascular system. The influence of RB® was evaluated at three levels: acute consumption, chronic consumption, as well as a combination of chronic and acute consumption.

Coronary autoregulation implies an intrinsic cardiac ability to maintain a relatively constant blood flow in response to a change in perfusion pressure when myocardial oxygen demand is constant [36]. The maximum rate of pressure change in the LV (dP/dt max) occurs at the end of the isovolumetric contraction and is used to estimate the inotropic properties of the myocardium [37], while the minimum rate of pressure change in the LV (dP/dt min) represents the relaxation rate (lusitropic properties of the myocardium) and reflects the maximum rate of pressure drop in the LV [38]. In our study, the increase in the level of dP/dt max and dP/dt min within the acED-T group was registered, when compared to the C-T group. In line with that, acute consumption of EDs contributed to an increase in SLVP, HR, and CF, while DLVP was lower in the acED-T group.

As for the impact of RB® and other EDs on cardiodynamics, the majority of previously published papers have focused on acute consumption and have been conducted on young, healthy people in a state of rest. As far as athletes are concerned, after consuming RB® and during their recovery phase following physical exercise, a significant increase in contractility of the left atrium and ventricle was registered [3]. It is believed that most of the biological effects of EDs are mediated by a positive inotropic effect [15], which is in line with our results. As for humans, it has been demonstrated that the acute consumption of 250 mL of RB® affects the increase in mean arterial pressure [22], but there are also studies that have shown no effect on systolic and diastolic arterial pressure at rest [28]. Additionally, in terms of the effect of EDs on the HR, non-homogeneous results were obtained (mostly with no effect or increase in the HR) [22, 28], but there was also a study published, showing how EDs influenced the reduction of the HR [39]. It has been shown that a higher dose of RB® (355 mL) affects the increase in systolic and diastolic arterial pressure and the increase in the HR [40], as well as that RB® at a dose of 500 mL affects the increase in the activity of the sympathetic nervous system [41]. The aforementioned can be explained by the effect of EDs on the increase in norepinephrine levels [42], which increases the HR and blood pressure, triggers the release of glucose from energy stores, and increases blood flow to skeletal muscles [16].

The dP/dt min level within the chED-T group was higher when compared to the C-T group, indicating a positive lusitropic effect of the EDs, while a decrease in DLVP was also registered. As it is the case with acute ED consumption, chronic consumption has also affected the increase in HR and CF. The fact that there was no significant difference in the dP/dt max level between the chED-T and the C-T group, as well as that SLVP was significantly higher in the chED-T group, can be interpreted as a negative influence of chronic ED consumption. Hypertension can cause LV hypertrophy, which is a risk factor for future cardiovascular events [43]. In pre-clinical studies, chronic use of EDs has mainly been evaluated through their effect on heart metabolism and, following our results, a negative effect on the heart of the rats has been registered [11, 44, 45]. Regular moderate exercise has beneficial effects on the heart [46], but our results show the chronic consumption of EDs can disrupt that effect.

Compared to acED-T, significantly lower levels of dP/dt max were observed in the ch + acED-T group, which may indicate slight depression in cardiac contractile force and systolic function. Moreover, when compared to the acED-T group, the lower values of dP/dt min (less negative) and CF were registered in the ch + acED-T group and, although they were not statistically significant, in combination with a significantly higher level of DLVP, they may be interpreted as mild changes in diastolic function. The obtained results suggest that EDs have a different effect on the cardiovascular system in chronic consumers when they acutely consume EDs and when it comes to occasional acute consumption. Given that the consumption of EDs by athletes is still a controversial topic, in terms of whether the benefits for improving the

performance are greater than the potential health hazards, the results describing how chronic consumption of EDs affects cardiovascular response in acute consumption can be useful for further research on this topic.

In acED-T and chED-T groups, when compared to the C-T, there was a significant increase in lipid peroxidation index level (estimated through the TBARS level), which indicates the deterioration of redox status. Furthermore, a significant increase in TBARS level was observed in the ch + acED-T group, when compared to the acED-T group, which suggests that in chronic consumers acute ED consumption continues to deteriorate redox status. The intense lipid peroxidation in biological membranes leads to the loss of fluidity, a decrease in the membrane potential, the increased permeability for H^+ and other ions and, in the end, a membrane rupture may also occur with the release of cellular content into extracellular space [47]. Our results are consistent with the increase in lipid peroxidation observed in the liver and brain of rats, after 14 days of using another commercially available ED [48]. In the chED-T group, an increased level of prooxidative species, O_2^- and H_2O_2, was also registered, when compared to the C-T. It is known that O_2^- and H_2O_2 affect the activation of the mitochondrial permeability transition pores, which leads to the loss of cytochrome C from mitochondria and the activation of caspases with the development of apoptosis [31, 49]. Generally, a larger amount of O_2^- reacts with NO, reducing its bioavailability and damaging endothelium-dependent vasodilatation [50]. A moderate-intensity training leads to a reduction in TBARS, O_2^-, and H_2O_2 [31] while, in our study, chronic ED consumption, in combination with moderate-intensity training, had the opposite effect and caused an increase in TBARS, O_2^-, and H_2O_2.

In the ch + acED-T group, when compared with the acED-T group, a significant decrease in the NO level was registered (estimated through the level of nitrite). It is known that atherosclerosis occurs due to the mechanism of vascular inflammation, which is defined by the increased production of ROS and due to the fact that endothelial dysfunction is characterized by the reduced production of NO [51]. NO is produced from L arginine and represents an important endogenous basal coronary tone regulator, while reactive hyperaemia and shear stress are a stimulus for the release of NO from the endothelium and the formation of vasodilatation. NO leads to the relaxation of the smooth muscles of coronary vessels, inhibits adhesion and platelet aggregation, inhibits leukocyte activation, and reduces the consumption of oxygen in the myocardium [52]. High glucose levels in EDs can be a factor that contributes to the platelet function damaging and the occurrence of endothelial dysfunction [22]. Hyperglycemia contributes to an increase in oxidative stress markers, and lipid peroxidation in erythrocytes is directly proportional to *in vitro* glucose concentration [53].

As it was already mentioned, EDs reduce endothelial function in humans at rest [22, 23, 54], and our results in rats show that this also applies to the physical activity of chronic consumers when they consume EDs acutely. On the other hand, acute administration of RB® at a dose of 250 mL and 355 mL has been shown to improve the endothelial function [12, 55], and this topic is an open field for further studies. Due to endothelial dysfunction, ED consumption is associated with an increased risk of myocardial ischaemia [56]. Previous evidence linking ED consumption with myocardial ischaemia is mainly based on case reports. The lack of randomized and prospective research is a major obstacle to the impossibility of establishing an unambiguous connection between excessive ED consumption and ischemia or myocardial infarction.

Conclusion

While the acute effects of EDs on the cardiovascular system are fairly clarified, chronic effects are much less studied and further research is suggested. The conclusion of our study is that acute administration of EDs had a positive inotropic effect, while chronic administration affected the isolated increase in SLVP, which could be considered the potentially negative impact of EDs. Chronic administration of EDs changed the cardiovascular response in acute consumption. Moreover, the prooxidative effect of EDs was observed. Due to the potential association of ED consumption with the onset of endothelial dysfunction and potential morbidity combined with physical exercise, further research is needed to clarify action mechanisms and significance of their effects, i.e. the correlations with clinical outcomes.

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