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RESEARCH

The effect of anthocyanin administration on redox balance in acute exercise: an experimental study

Akut egzersizde antosiyanin uygulamasının redoks dengesi üzerine etkisi: deneysel bir çalışma

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Abstract

Purpose: Due to the diverse biological activities attributed to its antioxidant, antidiabetic, anti-inflammatory, and cytoprotective properties, cyanidin-3-glucoside (Cy3G) has been widely studied. The present study was conducted to investigate the effects of Cy3G supplementation on redox balance disruption during acute exercise.

Materials and Methods: To this end, 28 male Balb-C mice were divided into four groups: control, exercise, Cy3G, and exercise+Cy3G. During the experimental period, the mice in the Cy3G and exercise+Cy3G groups were administered 5 mg of Cy3G per kg of body weight, while the control and exercise groups were fed standard chow. The mice were trained on treadmill for 10 min every day at speed of 10 m/min for 2 weeks. On the 15th day, an acute exhaustion exercise was applied. Then, all groups were sacrificed, and serum samples were taken to analyze the native thiol, total thiol, disulfide, and thiol-disulfide indices.

Results: The findings showed that the combination of Cy3G and exercise significantly increased native thiol levels and decreased disulfide levels compared to the other groups. However, it did not cause any change in total thiol levels. Native thiol were measured as 114.6±64.1, 106.4 ±57.9, 200.1±84.2, 262.1±105.9; Disulfide were determined as 253.2±71.6, 257.4±31.5, 213.8±44.8 and 188.4±32.2; Total thiol were 621.1±116.1, 621.2±100.1, 627.7±125.8 and 639.0±105.3 (respectively; control, exercise, Cy3G, and exercise+Cy3G).

Conclusion: Cy3G supplementation can potentially maintain redox balance during acute exercise by supporting antioxidant defense systems and reducing oxidative stress. The use of Cy3G may have a positive effect on preventing exercise-induced oxidative stress and acute fatigue.

Keywords:. Anthocyanin, oxidative stress, cy3g, thiol, disulfide

Öz

Amaç: Siyanidin-3-glikozit (Cy3G) antioksidan, antidiyabetik, antiinflamatuar ve sitoprotektif özelliklerine atfedilen biyolojik aktivitelerinin çeşitliliği nedeniyle geniş çapta araştırılmaktadır. Bu çalışmada akut egzersizde bozulan redoks dengesi üzerine Cy3G takviyesinin etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: 28 adet erkek Balb-C fare dört gruba ayrıldı: kontrol, egzersiz, Cy3G ve egzersiz+Cy3G. Deney protokolünün 1-14. günleri boyunca, Cy3G ve Egzersiz+Cy3G gruplarındaki farelere vücut ağırlığının kg'ı başına 5 mg Cy3G verildi. Kontrol ve egzersiz grubundaki fareler standart yem ile beslendi. Farelere akut tükenme egzersizinden önce 2 hafta boyunca 10 m/dak hızda her gün 10 dakika boyunca koşu bandında alıştırma egzersizi yaptırıldı. 15. günde ise akut tükenme egzersizi uygulandı. Ardından tüm gruplar sakrifiye edildi ve serum örneklerinde native thiol, total thiol, disülfit düzeyi ve thiol-disülfit indeksleri analiz edildi.

Bulgular: Egzersizle birlikte verilen Cy3G, native tiyol düzeyini diğer gruplara göre anlamlı düzeyde artırırken, disülfit düzeylerini düşürdüğü bulundu. Total tiyol düzeylerinde herhangi bir değişikliğe neden olmadı. Native tiyol 114.6±64.1, 106.4 ±57.9, 200.1±84.2, 262.1±105.9 olarak ölçüldü; Disülfid düzeyi 253.2±71.6, 257.4±31.5, 213.8±44.8 ve 188.4±32.2 olarak hesaplandı; Total tiyol ise 621.1±116.1, 621.2±100.1, 627.7±125.8, 639.0±105.3 idi (sırasıyla; kontrol, egzersiz, Cy3G ve egzersiz+Cy3G).

Sonuç: Akut egzersizde Cy3G takviyesi, antioksidan savunma sistemlerini destekleyerek ve oksidatif stresi azaltarak potansiyel olarak redoks dengesini koruyabilir. Cy3G kullanımı, spor branşlarında akut yorgunluğa neden olabilecek egzersiz kaynaklı oksidatif stresin önlenmesinde olumlu etki yaratabilir.

Anahtar kelimeler: Antosiyanin, oksidatif stres, cy3g, tiyol, disülfit

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INTRODUCTION

Over the years, there has been a noticeable increase in the use of supplements, such as dietary fiber, probiotics, vitamins, and polyphenols, to prevent and treat diseases[1]. Anthocyanins are among nature's most abundant polyphenol compounds and make up a significant portion of natural antioxidant supplements[2]. Anthocyanins are natural pigments found in fruits and vegetables[3]. Due to their diverse chemical structures, there are more than 500 different types of anthocyanins, with pelargonidins, cyanidins, delphinidins, peonidins, petunidins, and malvidins being the most common species[4,5]. Anthocyanins have been linked to diverse biological effects and potential health benefits in numerous studies[4].

Cyanidin-3-glucoside (Cy3G) is a crucial anthocyanin compound that naturally occurs in various purple-colored fruits, black rice, black beans, and purple potatoes. Studies suggest that Cy3G has potent antioxidant, anti-inflammatory, antidiabetic, and cytoprotective properties. It is especially useful in combating oxidative stress-induced disorder[6]. Cy3G exerts its protective abilities at the cellular level by neutralizing radical species and preventing oxidative damage. Its antioxidant capacity helps protect DNA and contributes to the stability of cellular components. Additionally, studies have shown that it has anti-inflammatory properties and may positively affect the immune system[7,8]. According to certain studies, the byproducts of Cy3G metabolism can control cellular signaling pathways and enhance cellular functions. Cy3G is thought to have the potential to promote metabolic well-being and prevent chronic diseases[9]. These discoveries offer a significant scientific foundation for understanding the biological functions of Cy3G.

Exercise is an indispensable element of human life, significantly affecting lifelong development and general health. In addition to building muscle mass, exercise helps in maintaining musculoskeletal function during aging. It also promotes cardiometabolic health, improves cognitive performance, and effectively contributes to the prevention and treatment of various health conditions, especially cardiovascular diseases, diabetes, metabolic disorders, neurological diseases, sarcopenia, osteoporosis, and cancer. Thus, exercise has various positive effects on health and improves quality of life[10].

Exercise causes molecular changes in almost every organ system, activating short- and long-term mechanisms to maintain and/or restore homeostasis. Reactive oxygen species (ROS) produced during exercise play critical roles in these complex processes. By activating cell signaling pathways, ROS mediate adaptation processes, such as skeletal muscle hypertrophy and mitochondrial biogenesis, and the induction of the endogenous antioxidant system[11].

Depending on intensity, duration, and type of exercise can lead to changes in redox balance, which is essential for the overall health of cells. Redox balance is the process of maintaining a balance between ROS production and elimination by antioxidant systems. Therefore, maintaining adequate redox balance during exercise is crucial for cellular health. Redox balance can be maintained by following a proper diet or using supplements [12]. In this study, we hypothesized that Cy3G supplementation during acute exercise may contribute to maintaining redox balance by strengthening antioxidant defense mechanisms.

Numerous biochemical techniques are used to evaluate changes in redox balance in biological systems. Thiol disulfide homeostasis is an important and widely accepted marker of these changes[13]. This study was conducted to investigate the effects of Cy3G supplementation, a flavonoid found in various fruits and vegetables, on the disruption of redox balance during acute exercise. This study was the first of its kind to explore the effects of Cy3G supplementation on redox balance during acute exercise. The study findings shed light on the potential use of Cy3G as a supplement that effectively improves redox balance in individuals who engage in acute exercise.

MATERIALS AND METHODS

Animals

The experimental animals were obtained from the Harran University Animal Experiment Application and Research Center and were housed in the same place throughout the experimental period. Specifically, male Balb/c mice (n = 28, age = 8 wk) were housed in a room with a 12:12 h light–dark cycle under a constant temperature of 22 ± 1°C and fed a standard laboratory mouse diet ad libitum with free access to water. The study protocol was approved by the Harran University Animal Experiments Local Ethics Committee (Date: 03.10.2023 No:

2023/006/03) and conformed to the Guide for the Care and Use of Laboratory Animals. The minimum sample size was 28 mice, calculated using the program G-Power (version 3.1.9.4), considering a type I error (alpha) of 0.05, a power (1-beta) of 0.8, and an effect size of 0.7. This study was conducted in the Harran University Faculty of Medicine Physiology Laboratory and the Harran University Animal Experiment Application and Research Center.

Experimental procedure

The mice were randomly divided into four groups of seven. The mice in Group I, the control group, were fed conventionally, without any exercise. Group II, the exercise group, was fed conventionally and made to undergo acute exercise. The mice in Group III, the Cy3G group, were orally administered Cy3G, without exercise. The mice in Group IV, the Cy3G + exercise group, were orally administered Cy3G alongside acute exercise. On days 1–14, the mice in the Cy3G and Cy3G + exercise groups received a gavage of 5 mg Cy3G per kg of body weight, and the mice in the control group and exercise group were fed a chow diet. On day 15, an acute exhaustion exercise was carried out using a treadmill (Ugo Basile, Animal Treadmill, ITALY) designed for rodents, with separate lanes for each animal and adjustable speeds and inclines. All animals in Group II and Group IV underwent familiarization exercises for 10 min at a speed of 10 m/min every day for 2 weeks. After the familiarization exercise, the mice ran at 10 m/min for 5 min, and the speed was increased from 10 to 32 m/min over 1 min [14]. Exhaustion was defined as the point at which a mouse spent more than 5 s on the electrically shocked grid without attempting to run again [15]. After the experimental procedure, all mice were sacrificed under deep anesthesia, and blood samples were collected for further evaluation of biochemical parameters. Serum samples were obtained after centrifugation and stored at -86°C in a deep freezer. All experimental procedures were carried out by the authors of the manuscript, who have PhD degrees in physiology and biochemistry. These authors have specific knowledge of the subject and related expertise, and they hold certificates for experimental animal.

Biochemical analysis

The serum samples were analyzed for thiol disulfide homeostasis, which is a pivotal gauge of the overall balance between oxidants and antioxidants within a system. For this analysis, an innovative automated method recently devised by Erel and Neselioglu was employed [16]. This technique relies on the reduction of dynamic disulfide bonds to reactive thiol groups in the presence of sodium borohydride, following which the levels of total thiol and native thiol are directly quantified. The computation of various parameters involves distinct mathematical expressions: The dynamic disulfide bond level equates to half the difference between total thiol and native thiol. The reduced thiol ratio is calculated as (native thiol/total thiol) × 100, the oxidized thiol ratio as (disulfide / total thiol) × 100, and the thiol oxidation–reduction ratio as (disulfide / native thiol) × 100. These parameters are also defined as thiol disulfide homeostasis indexes.

Statistical analysis

Statistical analyses were performed using SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA). Normality was assessed using the Shapiro–Wilk test. Normally distributed data were reported as mean ± standard deviation (SD) values, and non-normally distributed data were reported using descriptive statistical methods, such as medians (interquartile ranges). A comparison of continuous variables was performed using a one-way ANOVA for normally distributed data (for native thiol, total thiol, disulfide, reduced thiol, and oxidized thiol) and the Kruskal–Wallis test for non-normally distributed data (for disulfide / native thiol tests). If a significant difference was found between any two of the four groups, a pairwise comparison test (Tukey's post hoc test for normally distributed data and Bonferroni-corrected t-tests for non-normally distributed data) was performed. P values less than 0.05 were considered statistically significant.

RESULTS

All mice survived until the end of the experimental period. Statistical differences between the groups were found in the values for serum native thiol ($p=0.003$), disulfide ($p=0.037$), reduced thiol ($p=0.001$), oxidized thiol ($p=0.001$), and disulfide/native thiol ($p=0.004$). Although the total thiol values of Group IV were slightly higher than those of the other three groups, the differences were not significant ($p > 0.05$) (Table 1, Figure 1). Group IV had the highest rate of native thiol (262.1 ± 105.9 μmol/L), and its serum native thiol was significantly high compared to Groups I and II ($p<0.05$, $p<0.01$,

respectively). The dynamic disulfide value, which is an important indicator of oxidation, was the lowest (188.4 ± 32.2) in Group IV. The disulfide values of Group IV were significantly lower than those of Group II ($p < 0.05$), but no statistical difference was found when compared with the other groups ($p>0.05$).

Table 1. Comparison of thiol/disulfide homeostasis parameters among the groups

Characteristics	Group I (n=7)	Group II (n=7)	Group III (n=7)	Group IV (n=7)	*p value*
Native thiol (µmol/L)	114.6±64.1	106.4 ±57.9	200.1 ±84.2	262.1 ±105.9	**0.003**ab**
Total thiol (µmol/L)	621.1 ±116.1	621.2 ±100.1	627.7 ±125.8	639.0 ±105.3	0.989
Disulfide (µmol/L)	253.2 ±71.6	257.4 ±31.5	213.8 ±44.8	188.4 ±32.2	**0.037*b**
Reduced thiol (%)	19.4 ±11.6	16.4 ±6.9	31.4 ±10.0	40.0 ±12.5	**0.001**a,b**
Oxidized thiol (%)	40.2 ±5.8	41.7 ±3.4	34.2 ±5.0	29.9 ±6.2	**0.001**a,b**
Disulfide/native thiol (%)	183.5 (529)	281 (251)	98 (78)	66 (57)	**0.004**a,b**

Data with a normal distribution (Native thiol, Total thiol, Disulfide, Reduced thiol, Oxidized thiol) were presented as mean±SD, and data without normal distribution (Disulfide/native thiol) were presented as median (Interquartile range). A comparison of continuous variables was performed using one-way ANOVA for normally distributed data and the Kruskal–Wallis test for non-normally distributed data. Group I: Control, Group II: Exercise, Group III: Cyanidin-3-glucoside, Group IV: Exercise+Cyanidin-3-glucoside. Bold and italic values indicate statistically significant findings. *p<0.05, **p<0.01

a. Significance comparison between Group I and Group IV

b. Significance comparison between Group II and Group IV

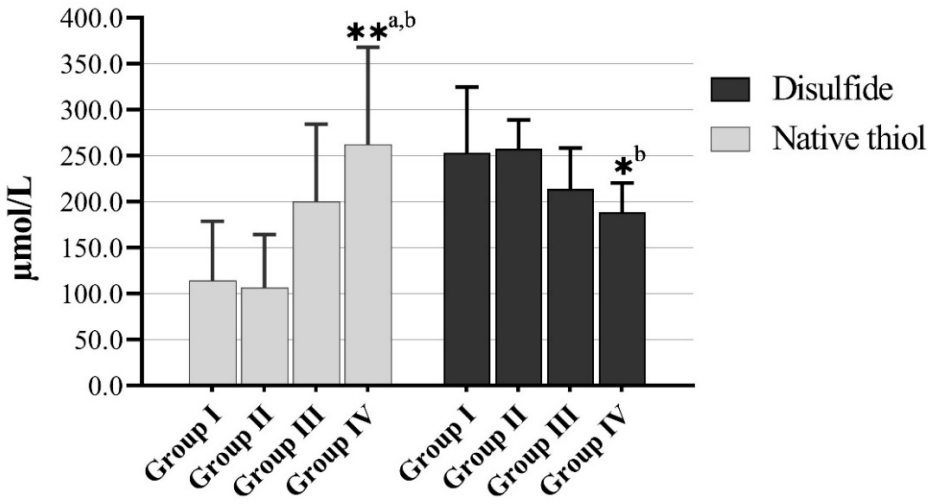

Figure 1. Comparison of native thiol and disulfide levels of experimental groups.

* *p* values less than 0.05 were considered significant. Group I: Control, Group II: Exercise, Group III: Cyanidin-3-glucoside, Group IV: Exercise+Cyanidin-3-glucoside.

a. Significance comparison between Group I and Group IV

b. Significance comparison between Group II and Group IV

The thiol disulfide homeostasis index also differed significantly between the groups. Group IV exhibited significantly higher reduced thiol values and lower oxidized thiol values and disulfide / native thiol ratios than the other three groups ($p < 0.01$ for both ratios when compared to both Group I and Group II). It should be noted that the statistical analyses of the oxidized and reduced thiol ratios and oxidation/reduction ratios were expected to be consistent across the groups.

DISCUSSION

In this study, we investigated the effects of acute exercise on redox balance in mice that received Cy3G supplementation via thiol disulfide homeostasis. This was the first study of its kind to examine the effects of Cy3G from the aforementioned point of view. The results highlighted the potential of Cy3G supplementation to maintain redox balance during exercise by strengthening antioxidant defense mechanisms and reducing oxidative stress. The findings provide valuable insights into the complex interplay between Cy3G and redox dynamics during acute exercise.

The redox state is a fundamental physiological balance mechanism that represents the continuous interaction between oxidants and antioxidants in cells. Oxidative stress describes a condition in which antioxidant defenses in the body cannot fully neutralize the harmful effects of free radicals and other ROS [17]. Mitochondria are the main endogenous sources of ROS, although others have also been identified. ROS are produced in mitochondria as part of aerobic metabolism. The oxidative metabolism process originating from mitochondria is tightly connected to ATP synthesis (oxidative phosphorylation) and is the main energy source for aerobic organisms[18]. The relationship between active energy expenditure and ROS production during exercise is quite complex.

The type of exercise can have positive or negative effects on an individual's redox biology depending on the specific characteristics and intensity of the training and the individual's baseline training level. It has been observed that regular moderate exercise reduces oxidative stress and has positive effects on health, whereas acute exercise is associated with increased oxidative stress. However, according to the hormesis theory, oxidative stress is necessary for the induction of endogenous antioxidant defense. This indicates that an organism's response to exercise occurs through adaptive mechanisms[19].

Various studies have been conducted to examine the role of ROS in regulating skeletal muscle activity. Skeletal muscle fibers constantly produce low levels of ROS; the production level increases during the contraction process. ROS exert a range of direct and indirect effects on muscle activity and contribute to skeletal muscle fatigue, especially during intense exercise[20]. ROS regulate both cell signaling and antioxidant gene expression and are thus crucial components of these processes. Therefore, balancing the amount of ROS produced is crucial for maintaining the positive effects of exercise and has the potential to positively impact skeletal muscle function and endurance, which are important factors that determine overall health and performance[21].

Plant bioactive compounds are commonly used to enhance human health. Among them, flavonoids stand out due to their potent antioxidant properties. Flavonoids have the ability to inhibit ROS formation by chelating metals and blocking the action of enzymes involved in radical chain reactions[22]. Anthocyanins constitute a subgroup of flavonoid compounds that are abundant in nature and make up a significant portion of dietary antioxidant supplements. The structures of anthocyanins determine their nutraceutical potential and, in turn, the specific physicochemical behaviors they exhibit in foods and biological systems[23].

Anthocyanins are responsible for the striking colors of fruits and flowers. These natural pigments are not limited to fruits and flowers but can also be found in other parts of plants, including leaves and stems. The pigmentation of a fruit is closely related to its anthocyanin concentration. The factors that determine the amount of anthocyanins produced in a fruit vary and include genetic and environmental factors, such as soil composition, light intensity, temperature, and humidity[24]. Anthocyanin-containing foods have attracted great research attention because they have emerged as promising treatment alternatives for tackling many chronic diseases with minimal reported side effects and have been adopted by consumers worldwide[24,25].

Cyanidin-3-glucoside is the most common type of anthocyanin found in edible fruits and exhibits strong antioxidant activity, which has been attributed to two hydroxyl groups in the B ring of its molecular structure[24]. Several studies have demonstrated the capacity of Cy3G to inhibit the NF-κB pathway[26,27]. Cy3G has also been observed to activate the Nrf2 pathway and to regulate the expression of antioxidant enzymes and cytoprotective proteins, such as superoxide dismutase, heme-oxidase, thioredoxin, catalase, glutathione S-transferase, and glutathione peroxidase[25].

An inaugural investigation of the potential benefits of anthocyanins with regard to exercise performance was carried out by Willems et al [28]. The findings showed that supplementation with anthocyanins led

to a notable reduction in the plasma lactate curve, consequently correlating with heightened exercise performance[28]. Another study that delved into the realm of exercise performance revealed that Cy3G supplementation resulted in extended swimming times and endurance among mice. This augmentation was linked to concurrent reductions in lactate levels and fatigue markers associated with physical activity[29].

The activation of the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) is vital for mitochondrial biogenesis in skeletal muscles and has been identified as a key mechanism influenced by Cy3G. Through the elevation of cyclic adenosine monophosphate levels, Cy3G induces the upregulation of PGC-1α in skeletal muscle, consequently enhancing exercise performance[29]. Despite these established connections, the interplay between Cy3G-induced adaptive mechanisms and the redox state remains unexplored. Exercise activates cellular signaling pathways via ROS, fostering adaptations such as mitochondrial biogenesis and inducing the endogenous antioxidant system in skeletal muscles[11]. The findings of our study align with those given in the literature, confirming that exercise (Group II) induces oxidative stress, as evidenced by elevated levels of disulfide, the earliest indicator of radical-mediated protein oxidation, and reduced levels of native thiol, an indicator of antioxidant activity.

In this study, the lowest disulfide value was detected in the exercise + Cy3G group (Group IV). It was even lower than the group that took Cy3G supplements and did not exercise. This suggests that the combination of exercise and Cy3G supplementation is more beneficial than Cy3G alone in reducing oxidative damage. The thiol disulfide homeostasis indices also supported our findings. Antioxidants are known to have free radical scavenging properties, due to which changes in antioxidant abundance may affect various cellular signaling pathways that are critical for exercise adaptation[30]. In a study regarding the effects of vitamins E and C, which are associated with high antioxidant activity, on acute strenuous running exercise, vitamin supplements taken alongside exercise were more effective than being sedentary and were reported to cause less oxidative stress[31]. This finding is supported by our observations of the exercise + Cy3G group in the present study.

Studies have shown that vitamins or phenolic compound supplements may be recommended to reduce the effects of heavy exercise[30,32,33]. In the present study, Cy3G supplementation prior to the acute exhaustion exercise induced redox homeostasis and could reduce the harmful effects of the exercise by changing the oxidant/antioxidant balance in favor of antioxidants. Although there are still many unanswered questions, it is evident that Cy3G usage helps prevent exercise-induced oxidative stress during sports that can lead to acute exhaustion. A limitation of our study is that the effects of Cy3G on other tissues, especially muscle and liver tissues, were not evaluated. Therefore, it is currently not possible to state that Cy3G prevents muscle damage one of the secondary effects of oxidative stress.

In conclusion, our investigation into the effects of acute exercise on redox balance in mice that received Cy3G supplementation through thiol disulfide homeostasis led to promising outcomes. The study demonstrated that Cy3G supplementation has the capacity to sustain redox equilibrium during acute exercise. This effect is attributed to the compound's ability to fortify antioxidant defense mechanisms while concurrently mitigating oxidative stress. These findings underscore the potential of Cy3G as a valuable adjunct in preserving redox homeostasis and offer insights into novel strategies for maintaining cellular integrity during acute physical exertion. Further research is warranted to elucidate the underlying molecular mechanisms and optimize the application of Cy3G supplementation in the context of exercise-induced redox perturbation.

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