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Original article

Effects of (+) - catechin + quercetin usage before exhaustion exercise on free radical and antioxidant enzyme levels

Erdil Durukan

School of Physical Education and Sport, Balıkesir University, Balıkesir, Turkey - E-mail: erdurukan@hotmail.com

Summary. *Study Objectives:* The aim of this study was to determine the effects of the use of (+) - Catechin + Quercetin for 10 days before an exhaustion exercise on free radical and antioxidant enzyme levels. *Methods:* The study was performed on 12 male Wistar rats (260-320 gr.) from the same family and animals divided into two groups as a control and experimental group. After the first exhaustion exercise, rats in the experimental group used (+) - Catechin + Quercetin in addition to the standard laboratory diet and they performed the second exhaustion exercise. In the experimental group, 20 mg/kg (+) - Catechin + Quercetin substances were dissolved in dimethyl sulfoxide and given 1 ml/kg while the control group received 1 ml/kg 0.05% dimethyl sulfoxide by gavage in addition to standard laboratory diet daily for 10 days. SOD, CAT, GPx, GST, and MDA levels were measured by spectrophotometer. The IBM SPSS Statistics 24.0 was preferred for the statistical analysis. The repeated measures two-factor variance analysis was used to determine the difference between control and experimental groups. *Results:* It was determined that antioxidant enzyme levels (SOD, CAT, GPx, and GST levels) in rats using (+) - Catechin + Quercetin for 10 days before an exhaustion exercise were higher than those of the control group. Despite that, it was determined that MDA levels in rats using (+) - Catechin + Quercetin for 10 days before an exhaustion exercise were lower than those of the control group. *Conclusion:* It can be said that the use of (+) - Catechin + Quercetin can reduce the amount of MDA which is the end product of lipid peroxidation in exercise and may create a protective effect against free radicals and increase the levels of antioxidant enzymes and strengthen the antioxidant defense systems of the cells and have a positive effect on exercise performance.

Key words: antioxidants, catechin, exhaustion exercise, free radicals, quercetin

Introduction

Physical activity and exercise have exhaustion impact mechanisms according to the type of physical activity and exercise besides protecting human health. In both human and animal experiments, their bodies have been found to have the ability to adapt to internal and external sources of stress. In addition, Yagmur et al. (2019) stated that exercise is a physical stressor that causes hormonal, metabolic, cardiovascular and immunological changes and that stress can affect the body during heavy exercise (1).

Although physical exercise has many beneficial effects on health, there are some findings showing that reactive oxygen species and free radical formation are increased particularly during heavy exercise, and that oxidative damage occurs in muscles, liver, blood and other tissues (2-6). The degree of the oxidative damage that can occur during physical exercise is determined not only by the production of free radicals, but also by the defense capacity of antioxidants. Superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) provide the first line of defense against reactive oxygen species produced during exercise. Therefore, exercise is thought to directly affect these enzymes (7).

It is known that exercise is a source of stress that causes the formation of oxidative stress by increasing the production of free oxygen radicals, whereas it in-

creases resistance against oxidative stress by affecting the activity of antioxidant enzymes. Cells have enzymatic and non-enzymatic defense mechanisms in order to minimize or eliminate the damage caused by free radicals particularly the ones produced through physical activity and exercise. Antioxidant enzymes such as SOD, CAT, selenium-dependent glutathione peroxidase (GPx) glutathione-S-transferase (GST) constitute the enzymatic defense mechanisms. Vitamin C, vitamin A, vitamin E, flavanoids, melatonin, uric acid, haptoglobin, albumin, cysteine, ceruloplasmin, transferrin, lactoferrin, ferritin, oxypurinol, ubiquinone (coenzyme Q10), bilirubin, mannitol, lipoic acid, and hemopexin are non-enzymatic defense mechanisms having antioxidant contents (8). Although Flavanoid (+) - Catechin and Quercetin, which are among the non-enzymatic defense systems, are present in different forms, they have a structure preferred both in experimental studies and as ergogenic support due to their antioxidant effects. In addition to superoxide, lipid alkoxyl, peroxyl and nitric oxide radical scavenging, iron and copper chelation, α-tocopherol regeneration functions of flavonoids and other plant phenolics, they have also a vasodilator, immunostimulant, antiallergic, estrogenic, antiviral (against HSV, HIV, Influenza, and Rhinoviruses) effects (9, 10). In a study by Hayek et al. (1997) on (+) - Catechin and Quercetin, which are known to have different mechanisms of action in the literature, it was reported that (+) - Catechin and Quercetins bind to LDL particles by ether bonding, reducing susceptibility to oxidation and aggregation (11).

Phenolic antioxidants interact with lipid oxidation in such a way as to rapidly give H + to lipid radicals. Its function is to break down lipid peroxyl and alkoxyl radicals and thus terminate the lipid peroxidation chain reaction (12). As is known, MDA occurs as the final product in the peroxidation of fatty acids containing three or more double bonds. The effect of Catechin on lowering lipid peroxidation level is associated with the fact that Catechin has direct free radical scavenging activity due to the excess of hydroxyl groups (13, 14), its function of breaking down free radical chain reaction by working synergistically with alpha-tocopherol to give hydrogen molecule for the regeneration of alpha-tocopherol (15), its preventing oxidation of low-density lipoproteins (16) and/or it's preventing free radical formation by binding iron and copper by acting as a chelator (17).

Yu et al. (2010) stated that flavonoid supplementation not only reduces free radical formation but also removes free radicals and improves endurance exercise performance by reducing muscle fatigue (18). Quercetin is an important flavonoid that prevents the formation of free oxygen radicals in cells and provides protection against lipid peroxidation. Kocabaş et al., (2008) found that plasma MDA levels of rats given Quercetin were lower than those of the control group ($p<0.05$) (19). Duarte et al. (2001) observed a decrease in MDA levels in rats after 5 weeks of use of Quercetin (20).

There are various studies in the literature on the effects of (+) - Catechin and Quercetin given through diet on antioxidant defense systems (SOD, CAT, GPx, and GST) to prevent damage caused by exercise-induced free radicals (21-28). Göktepe and Günay (2014) examined the effects of Quercetin on MDA, SOD, GPx and GST levels in rats. They found that Quercetin, which was administered through diet for 10 days, had a protective effect against free radicals by reducing the amount of MDA under exhaustion exercise conditions and strengthened the antioxidant defense systems of the cells by increasing the antioxidant enzyme levels (SOD, CAT, GPx, GST) (29). In another study, it was found that (+) - Catechin, which was applied as a diet for 10 days, decreased MDA level under exhaustion swimming exercise conditions and increased antioxidant enzyme levels (SOD, CAT, GPx, GST) (30).

In the literature, there is no study in which (+) - Catechin and Quercetin flavonoids that have different effect mechanisms are used together with diet and which investigates their effects on exhaustion exercise-induced lipid peroxidation and antioxidant enzyme levels (SOD, CAT, GPx, GST). Although the bioavailability of (+) - Catechin and Quercetin is relatively well documented, data on the effect of a relationship of these two flavonoids on their absorption and metabolism in dietary intake is still lacking. In addition, previous studies have reported that flavonoids may interact with each other, and these interactions may demonstrate effective competition against drugs for syner-

gistic antioxidant properties or metabolic enzymes (31-35). Therefore, the current study is important in terms of determining the effects of dietary intake of (+) - Catechin + Quercetin together on free radical and antioxidant enzyme levels during exhaustion exercise. In this connection, the purpose of the current study is to reveal the protective effects of (+) - Catechin + Quercetin by comparing MDA values and antioxidant levels as the final product of lipid peroxidation, which is the indicator of the effects of free radicals on membrane lipids after exhaustion swimming exercise, between the control and experimental groups. The hypotheses of the study are given below;

a) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise increases SOD level.
b) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise increases CAT level.
c) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise increases GPx level.
d) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise increases GST level.
e) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise decreases MDA level.

Materials and Methods

Characteristics and Diet of the Rats

Twelve male Wistar Albino rats weighing 260-320 g from the same family were used in the current study. The rats were randomly divided into two groups as the control group (n = 6) and the experimental group (n = 6). The rats were quarantined for 10 days, six rates in one cage before the experiment. All the rats were fed in special cages with standard laboratory diet and water. The rats were subjected to 12 hours of light and 12 hours of dark photoperiod at room temperature of 18-22 ° C. In addition, the temperature in the laboratory was 18-22 ° C and the relative humidity was 50 ± 10%.

Application and Experimental Design

After the 10 day-quarantine, all the rats performed exhaustion swimming exercises twice at different times (at an interval of 10 days) in a water tank of 80 x 60 x 60 cm^3. The exhaustion criterion was determined as the initiation of uncoordinated movements of the rats and/or their are remaining motionless for 10 seconds underwater (29). The water temperature in the water tank was 28°C. The rats performed all the exercises between 09:00 and 10:00 in the morning when they were full. In both exercises, rats were dried by a towel and then blood was taken from them.

First exercise: After the 10-day quarantine, all the rats performed the exhaustion exercise without using any substance other than the standard laboratory diet. After the exhaustion exercise, blood was taken from their hearts.

Second exercise: After the first exhaustion exercise, the rats in the experimental group used (+) - Catechin + Quercetin in addition to the standard laboratory diet and they performed the second exhaustion exercise. In the experimental group, 20 mg/kg (+) - Catechin + Quercetin substances were dissolved in dimethyl sulfoxide and given 1 ml/kg while the control group received 1 ml/kg 0.05% dimethyl sulfoxide by gavage in addition to standard laboratory diet daily for 10 days. Sigma-Aldrich brand (+) - Catechin, Quercetin, and Dimethyl sulfoxide were used throughout the experiments.

Biochemical Analyses

Preparation of Erythrocytes and Taking Blood Samples from Rats

In the experiment, blood was taken from the hearts of the animals after both exhaustion exercises into heparinized tubes with the help of a vacutainer. In the blood samples, erythrocytes were separated from the plasma through centrifugation at 1600 rpm +4 ° C for 5 min. They were then washed in cold 0.9% NaCl solution. The supernatant was carefully separated after each wash. Erythrocytes were suspended in pH 7.4 phosphate buffer. Hemoglobin concentration was determined according to the Drabkin (1946) method (36). Cell mixtures were stored at -20 ° C for 24 hours. Cells were detonated by forming an osmotic pressure difference with water and centrifuged at 2500 rpm. For 10 minutes. MDA level, SOD, CAT, GPx and GST activities of the obtained supernatants were measured by using a spectrophotometer (Shimadzu UV-1700, Japan).

SOD enzyme: In the determination of the total SOD levels, increasing absorbance was measured by

autoxidation of pyrogallol at 440 nm in alkaline medium using the Marklund and Marklund (1974) method (37). One unit total SOD activity was calculated as the amount of protein that caused 50% inhibition of autoxidation of pyrogallol. The SOD activity was determined as U / mg hemoglobin.

CAT enzyme: The activity of the CAT enzyme was determined with the method specified by Aebi 1984 (38). Decreasing absorbance indicating the breakdown of H_2O_2 was measured at 240 nm. Changes in absorbance per unit time were taken as a measure of CAT activity. Enzyme activity was given in U / mg hemoglobin unit.

GPx enzyme: The determination of the GPx level was made according to the method specified by Paglia and Valentine 1967 (39). Oxidation of NADPH to Nicotinamide-adenine-dinucleotide phosphate causes a decrease in absorbance at 340 nm, thus indirectly used in the determination of the activity of GPx. Hydrogen peroxide was added to this mixture to initiate the enzymatic reaction and absorbances were read at 340 nm for 3 minutes. GPx activity was calculated as the amount of NADPH spent per unit time and the specific activity of the enzyme was determined as U / mg hemoglobin.

GST enzyme: GST determination was performed according to the method developed by Habig et al. and 1-chloro-2.4-dinitrobenzene (CDNB) was used as the substrate for all isozymes of GST (40). The absorbance at 340 nm was read for the determination of the enzyme activity. GST specific activity was given as U / mg hemoglobin.

Determination of the Amount of MDA: In order to determine the amount of MDA; based on the method used by Ohkawa et al. (1979), the amount of MDA, the end product of lipid peroxidation reacting with thiobarbituric acid (TBA) at 532 nm, was measured (41). The absorbance of the mixture added with TBA was read at 532 nm on the spectrophotometer. The amount of MDA was determined as nmol/mg hemoglobin.

Ethical Approval

The current study was conducted in the Biology Laboratory, Faculty of Science and Letters, Gazi University and the ethical approval for the study was given by Gazi University, Animal Experiments Local Ethics Committee under the number G.Ü.ET-10.091.

Statistical Analysis

Statistical analysis of the data was conducted with the IBM SPSS Statistics 24.0 program package. The repeated measures two-factor variance analysis (2 groups X 2 times) was used for the analysis of the obtained data. In addition, the percent difference of the variables between two exhaustion exercises was calculated using the formula "Δ% = ((Post-test – Pre-test) / Pre-test) × 100" (42). The confidence interval was 95% and the level of significance was set at $p< .05$.

Results

In this section, the effects of 10-day use of (+) - Catechin + Quercetin on rats' free radical markers and antioxidant enzyme levels are presented.

It was found that there was a difference in SOD levels according to the measurement times (F= 49.339; p= .001). However, there was also a difference between the SOD levels of the groups. (F= 8.810; p= .014). In addition, the interaction between the groups and the measurement times was statistically significant (F= 34.111; p= .001). Accordingly, it was determined that an increase of 20.21% was observed in the SOD levels of the experimental group (Table 1).

It was found that there was a difference in CAT levels according to the measurement times (F = 71.361; p = .001). However, there was also a difference between the CAT levels of the groups (F = 15.398; p= .003). In addition, the interaction between the groups and the measurement times was statistically significant (F= 52.758; p= .001). Accordingly, it was determined that an increase of 41.16% was observed in the CAT levels of the experimental group (Table 2).

It was found that there was a difference in GPx levels according to the measurement times (F= 16.633; p= .002). However, there was also a difference between the GPx levels of the groups (F= 25.875; p= .001). In addition, the interaction between the groups and the measurement times was statistically significant (F= 18.091; p= .002). Accordingly, it was determined that

an increase of 49.95% was observed in the GPx levels of the experimental group (Table 3).

It was found that there was a difference in GST levels according to the measurement times (F= 27.455; p= .001). However, there was also a difference between the GST levels of the groups (F= 15.357; p= .003). In addition, the interaction between the groups and the measurement times was statistically significant (F= 15.357; p= .003). Accordingly, it was determined that an increase of 33.80% was observed in the GST levels of the experimental group (Table 4).

It was found that there was a difference in MDA levels according to the measurement times (F= 105.311; p= .001). However, there was also a difference between the MDA levels of the groups (F= 117.788; p= .001). In addition, the interaction between the groups and

Table 1. Effect of using 10 daily (+) - Catechin + Quercetin on SOD (U/mg)

Groups / Times	N	First Exercise	Second Exercise	Total	Δ%	F	p
		$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	6	593.40±23.73	605.23±24.74	599.31±8.74	1.99	8.810	.014*
Experimental	6	571.68±37.79	700.30±20.70	635.99±8.74	20.21		
Total	12	582.54±32.15	652.77±54.20		Interaction F= 34.111; p= .001**		
F= 49.339; p= .001**							

**p<0.05; **p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

Table 2. Effect of using 10 daily (+) - Catechin + Quercetin on CAT (U/mg)

Groups / Times	First Exercise	Second Exercise	Total	Δ%	F	p
	$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	249.47±27.36	257.37±24.66	258.16±8.71	3.17	15.398	.003**
Experimental	254.76±27.63	359.61±25.83	352.15±8.71	41.16		
Total	252.12±26.36	308.49±58.57		Interaction F= 52.758; p= .001**		
F= .71.361; p= .001**						

***p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

Table 3. Effect of using 10 daily (+) - Catechin + Quercetin on GPx (U/mg)

Groups / Times	N	First Exercise	Second Exercise	Total	Δ%	F	p
		$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	6	26.83±2.76	26.54±3.66	27.24± .98	-1.08	25.875	.001**
Experimental	6	27.65±3.91	41.46±5.04	34.00±1.27	49.95		
Total	12	27.24±3.25	34.00±8.82		Interaction F= 18.091; p= .002**		
F= 16.633; p= .002**							

***p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

Table 4. Effect of using 10 daily (+) - Catechin + Quercetin on GST (U/mg)

Groups / Times	N	First Exercise	Second Exercise	Total	Δ%	F	p
		$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	6	35.33±3.47	37.82±1.96	35.39± .96	7.05	15.357	.003**
Experimental	6	35.44±3.14	47.34±3.88	42.58± .89	33.80		
Total	12	35.38±3.15	42.58±5.78		Interaction F= 11.760; p= .006**		
F= 27.455; p= .001**							

***p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

the measurement times was statistically significant (F= 68.723; p= .001). Accordingly, it was determined that a decrease of 53.89% was observed in the MDA levels of the experimental group (Table 5).

Discussion

In addition to superoxide, lipid alkoxyl, peroxyl and nitric oxide radical scavenging, iron and copper chelation, α-tocopherol regeneration functions of flavonoids and other plant phenolics, they have also a vasodilator, immune-stimulant, antiallergic, estrogenic, antiviral (against HSV, HIV, influenza, and rhinoviruses) effects (12). A member of the flavonoid family, catechin is a flavonoid that is abundant in beverages such as fruit juices, red wine, and green tea and chocolate. In addition, recent studies have shown that catechin has anticarcinogenic, antimutagenic and hypodermic effects (43). Another member of the flavonoid family, quercetin is a bioflavonoid that is widely available in fruits and vegetables. Quercetin directly removes free radicals, inhibits lipid peroxidation, iron chelation, and strengthens antioxidant defense (44). Quercetin is known to reduce or prevent oxidative damage by improving antioxidant enzyme activity or by reducing lipid peroxidation (45-47). Changes in the levels of antioxidant enzymes in the blood as an indirect result of oxidative stress induced by exhaustion exercise are evaluated either alone or in combination with other oxidative stress indicators, MDA.

In the current study, the levels of free radical and antioxidant enzymes before giving (+) - Catechin + Quercetin (first exercise) and free radical and antioxidant enzyme levels after (+) – giving Catechin + Quercetin (second exercise) were measured in the experimental and control groups. In this context, it was found that there were significant decreases in MDA, which is the end product of lipid peroxidation, in the experimental group (rats consuming (+) - Catechin + Quercetin for 10 days) compared to the control group ($p<0.001$). It was also found that antioxidant enzyme levels (SOD, CAT, GPx, and GST) increased significantly after (+) - Catechin + Quercetin intake compared to the control group ($p<0.001$).

The effects of flavonoids on lipid peroxidation have been studied by many researchers and it has been shown that flavonoids significantly lower MDA levels (48-52). It was observed that cisplatin caused an increase in the amount of MDA in the rat kidney, whereas Quercetin decreased the increase in this lipid peroxidation (53). Çiftçi (2013) reported that Quercetin administered to rats has a reducing effect on MDA and may also prevent degenerative changes in the heart vessels (54). Hollman et al. (1995) found that antioxidant capacity was significantly higher and lipid peroxidation was inhibited in rats fed with a diet containing 0.2% Quercetin compared to the control group (55). In another study, which tried to determine the antioxidative efficacy of Quercetin, they found that 2 weeks of Quercetin administration caused a decrease in MDA level in rats (56). Göktepe and Günay (2014) also concluded that Quercetin application had a protective effect against free radicals in rats by reducing the amount of MDA, the end product of lipid peroxidation (29).

Catechin, another flavonoid, is known to exhibit protective behaviour against pathologies such as cell toxicity, cancer development, and free radical oxidation. In a study, it was reported that Catechin administration in rats significantly reduced the consumption of α-tocopherol and the accumulation of lipid perox-

Table 5. Effect of using 10 daily (+) - Catechin + Quercetin on MDA (nmol/mg)

Groups / Times	N	First Exercise	Second Exercise	Total	Δ%	F	p
		$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	6	18.94±1.00	17.87±1.36	18.89± .35	-5.81	117.788	.001**
Experimental	6	18.84±1.37	8.71±1.01	13.29± .35	-53.89		
Total	12	18.89±1.15	13.29±4.92		Interaction F= 68.723; p= .001**		
F= 105.311; p= .001**							

***p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

ides in plasma (57). According to the result of the current study, the use of (+) - Catechin and Quercetin is similar to that of flavonoids in the literature on MDA.

Antioxidants remove free radicals in the environment. For this purpose, SOD, CAT, and GST are in antioxidative defense (58). Quercetin has a wide range of therapeutic properties such as antioxidant, antitoxic, anti-cancer, anti-variant, anti-diabetic, anti-inflammation, cardiovascular effects, which are particularly beneficial to health (59-65). It is also known that catechins act as antioxidants by removing free radicals from the environment (66). Indeed, Phachonpai et al. (2010) reported that quercetin administered to rats increased SOD, CAT and GPx enzyme levels (27). In another study, it was found that Quercetin, which was administered for 2 weeks, increased SOD, CAT, and GPx enzyme levels in rats (56). Gargouri et al. (2011) reported that quercetin administration increased SOD and CAT enzyme levels in human lymphocytes (67). Bu et al. (2011); in their study investigating the protective effect of Quercetin against cadmium-induced oxidative toxicity in testicular germinative cells of mice, reported that test animals were administered cadmium (4mg/kg/day) and quercetin (75mg/kg/day) for two weeks and at the end of the study antioxidant enzyme activities (SOD and GSH-Px) were significantly improved and lipid peroxidation and hydrogen peroxide production were controlled significantly (68).

In relation to the effects of Catechin on antioxidant enzyme levels, Chan et al. (2002) reported that Catechin is an effective antioxidant that increases SOD activity in rat astrocytes (69). In another study, it was concluded that catechin increased the levels of SOD, GPx, and CAT enzymes (70). Sadowska-Krępa et al. (2008) stated that exercise type and intensity may affect the response of antioxidant defense systems and as a result, they found that red grape extract with Catechin content slightly increased antioxidant enzyme levels (SOD, CAT, and GPx) in the experimental group after interval swimming test compared to the control group (71). Yu et al. (2010) examined CuZn-SOD and GPx activities in order to determine the effects of Cynomorium Songaricum as a flavonoid extract with Catechin content on swimming resistance and free radicals of rats and reported that the group's given flavonoid extract have higher antioxidant enzyme levels than the group not given (18). The results of the studies in the literature showed that Catechin and Quercetin increased antioxidant enzyme activities. The results of the current study are similar to the ones reported in the literature in terms of the effects of Catechin and Quercetin on antioxidant defense mechanisms.

Conclusion

It was found that at the increasing free radical level as a result of exhaustion exercise, the use of (+) - Catechin + Quercetin together resulted in a decrease of 36.79% in the level of MDA, the end product of lipid peroxidation. In addition, it resulted in an increase of 18.48%, 36.41%, 56.52%, and 26.50% in the levels of antioxidant enzymes SOD, CAT, GPx, and GST, respectively. This result shows that (+) - Catechin + Quercetin administered during exhaustion exercise will decrease free radical enzyme levels and increase antioxidant defense enzyme levels. Considering the results of the study, it is recommended that (+) - Catechin and Quercetin can be taken together through diet by individuals engaged in exhaustion exercise. In this way, the athletic performance of the individuals doing a exhaustion exercise can be enhanced.

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Correspondence:
Erdil Durukan
School of Physical Education and Sports, Balıkesir University, Balıkesir, Turkey
E-mail: erdurukan@hotmail.com