EFFECT OF SIX WEEKS OF QUERCETIN SUPPLEMENTATION ON EXERCISE PERFORMANCE AND MUSCLE DAMAGE IN MALE BOXERS

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Abstract
The aim of this study was to determine the effects of six weeks of quercetin supplementation on exercise performance, muscle damage indices and body muscle in boxer. It included 20 voluntary male athletes. The sportsmen were separated into two groups as the control group (n = 10) and Quercetin group (n = 10). The athletes were supplemented with 500 mg quercetin for six weeks before their exercise programs. Before (B.T.) and after (A.T.) the training, blood samples were taken from athletes in order to determine aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glutamyl transpeptidase (GG), lactate dehydrogenase (LDH), Creatine kinase (CK) enzyme activities and VO2max. According to the findings, there was no significant difference detected between the groups when they were compared before exercise; however, there were a decrease in the VO2max levels of the control group (P<0.01) and a significant increase in the VO2max levels of quercetin group (P<0.001) when they were compared after being given quercetin. In addition, significant increase (P<0.01) were obtained in the levels of AST, ALT, ALP, GG, LDH and CK of the control group compared to those of the quercetin group. As a result, Trial group AST, ALT, ALP, DD, while the LDH and CK levels are the liver and muscle enzyme activity compared to controls low VO2max levels was concluded to be due to the use of Quercetin be higher.

Key words: quercetin, exercise performance, muscle damage

1. INTRODUCTION
The flavonoids are comprised of a large group of low molecular weight polyphenolic secondaryplant metabolites which are important to the health of animals and humans. Dietary exposure to flavonoids is not insignificant (Petruška et al. 2013). For instance, the average Western diet contains approximately 1 g.day-1 of mixed flavonoids (Hollman and Katan, 2009). Ingestible flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers, roots, bark, tea, wine and coffee. They are prominent components of citrus fruits and other food sources (Jellin et al., 2003). Their effects on a variety of inflammatory processes have been reviewed (Wright et al., 2012). They are also potent antioxidants and possess significant vitamin C-sparing activity (Mc Cullough et al., 2012).

There are also findings that the formation of free radicals and reactive oxygen species (ROS) increases especially during heavy exercise, and oxidative damage forms in muscles, liver and other tissues (Konig et al. 2001; Urso et al. 2003). The heavier the exercises are, the freer radicals form (Göktepe and Günay 2014). In connection with the oxygen consumption, increased free radicals are neutralized by a defense mechanism including both enzymatic and non-enzymatic antioxidants. Quercetin's action mechanisms with regards to free radicals are diverse. Quercetin hydroxyl radical shows the highest level of anti-radical characteristics against peroxyrad and superoxyde anion when compared to other flavonoids. Quercetin inhibits superoxyde anion generation through xanthine oxydase, and cleans singlet oxygen as well as hydroxyl radicals (Casagrande et al. 2006).

During exercise, oxygen and energy needs of muscles increase together with the necessity of removing metabolites and carbon dioxide. As a result of the increases in such necessities, chemical, mechanical and thermal stimuli cause changes in metabolic, cardiovascular and respiratory functions. With lifting, quite sharp changes occur within the organism. Changes such as muscular contractions, respiration, heart rate, sweating, energy use, enzyme actions etc. impact homeostasis (Burton et al., 2004). During muscular activity, O₂ use in active muscles increases, and together with this, nutrients
are needed for satisfying the energy need of active muscles. More waste products are formed with the increase of metabolic activities. Besides, respiratory rate increases too. Functional state of respiratory system is classically determined through the measurement of pulmonary volume and capacity (Atan et al, 2013). Moreover, an increase occurs in O₂ consumption rate in tissues, which is called VO₂max. Through the exercise taken, sportsmen supply their body with much more oxygen than they actually need. Therefore, what is important is to increase the usability of oxygen, or in other words, VO₂max through the exercise program applied (Tamer, 1995). While it is known that regular exercise programs have positive impact on respiratory and circulatory systems, studies conducted on respiratory parameters of sportsmen bring about different opinions. While some of these studies indicate that intensive physical exercises have an increasing impact on the respiratory parameters (Açıkada, 1982; Gelecek et al., 2000), some others point out that such an increase occurs depending on the developmental characteristics of the group of age (Ergen, 1983; Hagberg et al, 1988).

Another indicator used to assess muscle damage is the increase in serum levels of muscle enzymes such as AST, ALT, ALP, GGT, LDH and CK. High enzyme levels are the indicator of the fatigue, damage and increase that occur in concentrations of big tissue structures such as liver and skeletal muscle. Different studies indicated that these enzymes increase after exercise-related muscle damage and 72 h after the exercise, enzyme levels turn back pre-exercise basal levels (Diaz et al., 2010; Schneider et al., 1995). After a variety of exercise programs and sports competitions of athletes in different sports branches according to some studies, increases in liver enzymes has been reported. (Lenaerts et al., 2005; Haralambie 1973). Some studies showed that changes in liver enzyme levels (Marcos et al., 2008; Lippi et al., 2011). Through this study, it was aimed to this study was to determine the effects of six weeks of quercetin supplementation on exercise performance, muscle damage indices and body muscle in boxer.

2. MATERIALS AND METHODS

2.1. Participants

This study included 20 voluntary licensed male boxers of 61-64 kg weight and 161-170 cm height between the ages of 18 and 21, who were documented to have no inconvenience for exercising with doctor's report. Conducted in line with the relevant directive specified in Helsinki Declaration. The voluntary sportsmen were informed in detail with regards to the rules to be followed, the supplementary materials to be used as well as the tests to be carried out within the scope of the study. Applications were performed at the Physiology laboratory of Kafkas University, Institute of Health Sciences, and the study protocol was approved by the Local Ethics Committee of Kafkas University Medical Faculty (Decision No. 80576354-050-99/05).

2.2. Exercise Protocol

The boxers having participated in the study were separated into two groups. The first group was evaluated as the control group, while the second group was evaluated as Quercetin group. An exercise program of two hours and 80-90% intensity was applied to the sportsmen three times a week for one month. It was ensured that the sportsmen did not take any medicine within 15 days before the test, which may affect antioxidant defense, and their diets were standardized. Quercetin group was supplemented with 500 mg Quercetin for 30 days, 15 mins. before exercises.

2.3. The Exercise Program Applied

The program was begun with 20 minutes of general warm-up exercises. After warming up, the sportsmen had 3 rounds of gloves or punching bag exercise, 1 round of shadow boxing as well as 1 round of rope jumping and walking. Using all box techniques, the sportsmen repeated the techniques stated below in 3 sets with maximal load. Techniques: having grade position and walking forward, backward, rightward and leftward in grade position as well as adjusting distance. The exercise was finished with the following: left and right crosses in the chin, defense against left and right crosses, left and right crosses in the chin and body and defense against them, left and right hooks in the body and defense against them, shadow boxing using the left and right hooks learned.
2.4. Collecting the blood samples and analyses

Blood samples were drawn from all the sportsmen for two times, one being before the exercise and the other after the 30th day of the exercise. After the blood samples drawn centrifuged at 4000 rpm for 15 mins. In cooling centrifuge machine, the part swith plasma left at the top were put into polypropylene tubes, and stored in deep freezer at - 20 °C until their AST, ALT, ALP, GGT, LDH and CK analyses were carried out. AST, ALT, ALP, GGT, LDH and CK blood samples were analyzed in Medicine Faculty Laboratory of Kafkas University.

2.5. Measurement of VO2max Values

12 minutes run-walk test (Cooper), an indirect method, was carried out in order to determine maximal oxygen consumption (VO2max). The results were calculated by multiplying each tour distance (400 m) by the number of tours run and adding the distance of the incomplete tour (in meters) to the result of multiplication. VO2max values were determined by Balke formula (Balke, 1961). VO2max ml/kg-min. = 33.3+(X-150)x 0.178 ml/kg-min. X= the distance run in 1 minute (Tamer, 2000).

2.6. Statistical Analysis

The statistical analysis of the data was conducted on the SPSS 17.0 software. The data were expressed as mean ± standard deviation. Comparisons were performed between the variables among groups through the t-test and between in-group variables through the paired t-test. P values lower than 0.05 were regarded as significant.

3. RESULTS

The study was participated by 20 elite boxers with ages of 18-21 years, heights of 161-170 cm, body weights of 61-64 kg and Body Mass Index (BMI) 21-23 kg/m2 (Table 1).

<table>
<thead>
<tr>
<th>Parameters (n=20)</th>
<th>Control group Mean±SD</th>
<th>Quercetin group Mean±SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>19.00± 1.05</td>
<td>18.90± 1.10</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.50± 2.83</td>
<td>167.20± 3.22</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.70± 1.15</td>
<td>63.00± 1.05</td>
<td>.081</td>
</tr>
<tr>
<td>BMI (kg/m)</td>
<td>23.00± 0.94</td>
<td>22.30± 0.67</td>
<td>.089</td>
</tr>
</tbody>
</table>

p>0.05* Body mass index (BMI)

There was no statistical difference between the groups in terms of physical characteristics. There was no significant difference detected between the groups when they were compared before exercise; however, there were not show a significant difference the VO2max levels of the control group and a significant increase in the VO2max levels of quercetin group (P<0.001) when they were compared after being given quercetin (Figure 1).
Maximal Oxygen Consumption (VO2max)

**Figure 1.** Changes in the VO2max Values of the Groups

In addition, which were supplemented with quercetin and then exercised, were observed before and after the exercise; significant increase (P<0.01) were obtained in the levels of AST, ALT, ALP, GG, LDH and CK of the control group compared to those of the quercetin group. However, p<0.01 decrease was found in LDH and CK levels of experimental groups and p<0.05 decrease was found in AST, ALT values with Quercetin usage. On the other hand, no significant difference of ALP and GGT levels was found in control and Quercetin groups (Table 2).

**Table 2.** The mean and standard deviation (±SD) values of liver enzyme activity levels of groups before and after training

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control Group</th>
<th>Quercetin Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-exercise</td>
<td>Post-exercise</td>
</tr>
<tr>
<td></td>
<td>X± SD</td>
<td>X± SD</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>147.0 ± 9.3</td>
<td>414.8 ± 28.4**</td>
</tr>
<tr>
<td>CK (IU/L)</td>
<td>11.84 ±4.68</td>
<td>30.4 ± 12.30**</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>32.2 ± 2.16</td>
<td>35.05 ± 2.82**</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>27.03 ± 7.04</td>
<td>29.77 ± 7.05**</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>25.55 ± 11.70</td>
<td>25.85± 11.90</td>
</tr>
<tr>
<td>GGT (mg/Dl)</td>
<td>12.15 ± 4.75</td>
<td>11.55 ± 4.30</td>
</tr>
</tbody>
</table>

** p<0.01, * p<0.05, Before Exercise (Pre-exercise), After Exercise (Post-exercise), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Glutamyl Transpeptidase (GGT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK).
4. DISCUSSION AND CONCLUSION

The aim of this study was to determine the effects of six weeks of quercetin supplementation on exercise performance, muscle damage indices and body muscle in boxer. According to our findings; There was no significant difference detected between the groups when they were compared before exercise; however, there were not show a significant difference the VO2max levels of the control group and a significant increase in the VO2max levels of quercetin group when they were compared after being given quercetin. Flavonoids comprise a large group of plant metabolites, 6,000 of which have been identified to date. Some studies have shown the increased aerobic performance and maximal oxygen consumption (VO2max) and therefore fitness following quercetin intake as a result of elevated number of intracellular mitochondria caused by the flavonoid (Williamson and Manach, 2005). Human performance studies using antioxidant supplementation have been frequently carried out in a maximal exercise capacity such as a long duration race or via maximal oxygen testing. We found only two studies with a treatment time of at least 6 weeks where the effects of quercetin on endurance performance in humans have been evaluated (MacRae and Mefferd, 2006). MacRae and Mefferd found a 3% improvement in cycle time trial performance following 6 weeks of supplementation. Study by Bigelman et al (2010) found no significant difference in VO2peak or anaerobic performance measures with 6 weeks of quercetin supplementation in moderately trained military personnel.

Davis et al. (2009) found that endurance capacity with quercetin supplementation in mice was associated with positive changes in markers of mitochondrial biogenesis in just 7 days of supplementation. It is possible that flavanols, including quercetin, may enhance mitochondrial biogenesis in humans, hence improve aerobic performance. In another study, quercetin supplementation did not enhance performance through peak oxygen consumption (Scholten and Sergeev, 2013). Ganio et al. (2010) found that 5 days of quercetin supplementation did not improve VO2max in untrained, sedentary individuals. During exercise, oxygen and energy needs of muscles increase together with the necessity of removing metabolites and carbon dioxide. As a result of the increases in such necessities, chemical, mechanical and thermal stimuli cause changes in metabolic, cardiovascular and respiratory functions. Contrary to our findings, Davis et al. (2010) found a significant improvement in VO2max and endurance performance using human subjects during a cycling bout with quercetin supplementation for 7 days. The authors hypothesized that the change in performance was related to mitochondrial biogenesis, reduction of reactive oxygen’s species preventing fatigue, and/or the psychostimulatory effect, which may also delay fatigue. The improved physical performance caused by quercetin reported by some studies might be attributable to the decreased membranes in muscles, which in turn reduces the negative and exhaustive effects of excessive oxygen radicals during physical activity (MacRae and Mefferd, 2006). Proving this hypothesis to be right would mean quercetin to be able to reduce muscular damage and soreness, as well as neuromuscular dysfunction following exercise. However, quercetin has been reported to have contrasting antioxidant effects.

On the other hand, high enzyme levels are the indicator of the fatigue, damage and increase occur that in concentrations of big tissue structures such as liver and skeletal muscle. Changes in concentrations CK, AST, LDH, ALP, GGT and ALT were examined so as to assess the muscle damage (Hazar, 2004). In addition, another indicator used to assess muscle damage is the increase in serum levels of muscle enzymes such as AST, ALT, ALP, GGT, LDH and CK. High enzyme levels are the indicator of the fatigue, damage and increase that occur in concentrations of big tissue structures such as liver and skeletal muscle. Different studies indicated that these enzymes increase after exercise-related muscle damage and 72 h after the exercise, enzyme levels turn back pre-exercise basal levels (Diaz et al., 2010). According to our findings; which were supplemented with quercetin and then exercised, were observed before and after the exercise; significant increase were obtained in the levels of AST, ALT, ALP, GG, LDH and CK of the control group compared to those of the quercetin group. However, decrease was found in LDH and CK levels of experimental groups and decrease was found in AST, ALT values with Quercetin usage. On the other hand, no significant difference of ALP and GGT levels was found in control and Quercetin groups.
Studies indicated that Quercetin taken before exercise reduces fatigue and increases level ALT ve AST (Yabe et al. 2001; Crockett et al. 2006). The work we have done quercetin was found to cause a significant reduction on the ALT and AST levels. These findings support the findings of the present research. In our study, liver cell damage that show that the level of liver enzymes ALT AST to be significantly lower in the experimental group and also show us that tissue damage is less than the group which quercetin. The increase in ALT and AST levels and that this increase is ischemia reperfusion injury caused by free radicals may be due to tissue damage caused postulated. Also, in another study the use of Quercetin before exercise leads to a clear decrease in CPK, LDH, AST, ALP levels during the exercise. However, control group produced severe liver injury by significantly increasing the serum levels of ALT, AST, GGT and LDH compared with that of the experimental (Surapaneni and Jainu, 2014). In another study AST and LDH values increased significantly in males, while only AST values increased significantly in females post-exercise (Uadia et al., 2016).

5. CONCLUSIONS
Long-term and intense s Boxing trainings lead to the increase in CK, LDH, AST and ALT enzyme activities due to excessive muscle activity and oxidative damage occurs as a result. Trial group AST, ALT, ALP, DD, while the LDH and CK levels are the liver and muscle enzyme activity compared to controls low VO2max levels was concluded to be due to the use of Quercetin be higher. We showed that the positive effects of quercetin on liver and muscle damage in this work we have done. During the exercise with the use of pre-exercise Quercetin the basis of these findings and the results may be effective in the prevention of muscle damage that can develop in sonrs it can be assumed.

REFERENCES


