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Effects of Quercetin Supplementation on Exercise Induced Inflammation and Immune Cell Changes After Exhausting Swimming in Adolescent Girls

Mandana Gholami [1,*] and Mahia Ardestani [1]

[1] Department of Physical Education and Sport Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

[*] *Corresponding author*: Department of Physical Education and Sport Science, Science and Research Branch, Islamic Azad University, Sattary High Way, University Square, Hesarak Blv., Tehran, Iran. Tel: +98-912149186, Fax: +09-2144865160, Email: man.gholami149@gmail.com

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Abstract

Background: It is suggested that quercetin has anti-inflammatory properties and can improve endurance performance, but study results are contradictory especially in human models. Therefore, the purpose of the present study was to evaluate the effect of quercetin supplementation on TNF-α, lactate and immune cell responses after one session of exhausting swimming.
Methods: 20 trained (at least 3 years' history of swimming training) female adolescent swimmers with average age of 15.1 $\pm$ 0.21 yrs and BMI, 21.05 $\pm$ 2.3 kg/m^2 were divided in two groups including placebo (n = 10) and quercetin (n = 10) groups. Participants in the quercetin group consumed 1000 mg of quercetin supplement daily for two weeks. After two weeks, all participants took part in one exercise session as swimming with 80 - 85 percent of maximum heart rate until exhaustion. Blood samples were collected at three times (baseline, pre and immediately after exercise) and TNF-α was measured by ELISA method. data were analyzed by SPSS-24 software using repeated-measures ANOVA and paired sample *t*-test. $P < 0.05$ was considered as significant difference.
Results: Results indicated that immediately after exercise, TNF-α response was significantly lower in quercetin compared to placebo group (P = 001). Lactate immediately after exercise increased in two groups (P = 001) but the difference between two groups was not significant ($P > 0.05$). Moreover, the quercetin supplement caused change in immune cell response in which the difference in leukocytes and monocytes were significant ($P < 0.05$) compared to placebo group.
Conclusions: It seems that quercetin can exert anti-inflammatory function after exhausting swimming. Therefore, quercetin can be consumed by athletes to attenuate exercise-induced inflammation.

Keywords: Inflammation, Supplement, Exercise

1. Background

Cytokines are a heterogeneous group of regulatory proteins with low molecular weight that regulate intensity and duration of immune responses. Furthermore, they control the activation, proliferation and differentiation of a diverse range of cells (1). Cytokines are produced by different body tissues as a family of intracellular peptides that affect inflammatory and immune responses. These secretory factors (cytokines) are classified as pro-inflammatory (such as interleukin 1-α (IL-1α), IL1-β, tumor necrosis factor α (TNF-α)) and anti-inflammatory (such as IL6 and IL10), which interact together to regulate inflammatory situations (2). TNF-α is a cytokine with pro-inflammatory properties that have important roles in apoptosis, cell proliferation and adaptive and innate immunity. These functions of TNF-α are mediated by binding this cytokine to type1 and type2 receptors (3). According to a previous study, exercise can cause transient alteration in circulating cytokine levels, specially an increase in IL-6 levels. This change can be related to anti-inflammatory properties of exercise (4). Acute exercise causes rapid adaptation in the cardiovascular, endocrine, musculoskeletal and CNS systems, therefore leads to physiological disturbances (5). It has been reported that strenuous exercise is associated with an increase in levels of inflammatory mediators; but simultaneously levels of anti-inflammatory cytokines lead to restriction of the magnitude and duration of the inflammatory response to exercise (6). TNF-α plays an important role in host response following acute exercise. Some study indicated that circulating levels of TNF-α increase significantly after acute exercise (7). However, another study reported

that TNF-α can't be affected during and after exercise (8). Therefore, the result about change in TNF-α circulating levels after exercise is controversial, with most researchers showing increase in TNF-α levels after strenuous exercise (9). It seems that elevation in pro-inflammatory markers is related to increasing exercise intensity as well as exercise duration, recruited muscle mass, and endurance capacity of individuals (10, 11). In addition to inflammatory and anti-inflammatory marker changes, acute or chronic exercise affects immune function. Its reported that regular exercise with moderate intensity improves immune function, but high volume intensive exercise training can result in increasing infection in athletes and attenuate immune function. Moreover, it is suggested that immune response to acute exercise session is related to intensity and duration of exercise (12).

Different nutritional supplements consist of N3-polyunsaturated fatty acids, glutamine, bovine colostrum, and many types of antioxidants including vitamins C and E have been studied because their potential roles in countering with exercise-induced inflammation (13). Among different supplements, quercetin is receiving increasing attention because of its anti-inflammatory and antioxidant functions (14). Quercetin is known as one of the more bioactive flavonoids which exist in many foods including apples, onions, grapes, black tea etc (15). In addition to the previously mentioned futures of quercetin, cardioprotective and especially ergogenic properties of quercetin have been taken into consideration by sports scientists. In this way, quercetin's role in improving endurance capacity was demonstrated (16). Research has shown that ingestion of pure quercetin (1000 mg/day) for five weeks lead to decrease in illness rates. However, quercetin supplementations did not attenuate exercise induced inflammation and immune dysfunction (17). Furthermore, Konrad et al. (18) reported that acute ingestion of quercetin-based supplements couldn't attenuate post exercise increase in inflammatory markers such as TNF-α and IL-1Rβ. In contrast, Nieman et al. (19) indicated that three-week ingestion of quercetin reduced post exercise inflammatory mediators such as IL-6 and TNF-α after a 3 hour cycling bout compared to placebo group. It seems that the varying reported results are related to difference in dose, duration and components of quercetin supplement in addition to type, intensity and duration of administrated exercise. We hypothesized that short term quercetin supplementation (two weeks) attenuates exercise related inflammation following exhausting swimming exercise. Previous studies have investigated the effect of quercetin supplementation on exercise induced inflammation after different exercise protocols (such as running, cycling, etc) especially in male adults and following exercise sessions with given time. However, in the present study we determine effects of quercetin supplementation after an exhausting swimming exercise session in adolescent girls.

2. Objectives

In fact, in the present study for the first time, the response of TNF-α, lactate and immune cells are studied after two-week quercetin supplementations in adolescent swimmers following exhausting high intensity swimming.

3. Methods

3.1. Participants

Twenty moderately trained adolescent swimmer girls (average age 15.1 $\pm$ 0.21 years, weight 51.8 $\pm$ 3.4 kg and height 155.1 $\pm$ 3.7), age ranging 14 to 16 from Tehran, who were not taking medications or any vitamin and antioxidant supplements exceeding 100% of RDA that don't have any physical and physiological problem and limitations for taking part in strenuous exercise were recruited for participation in the present study. The number of participants was chosen according to previous studies (20). Written, informed consent was obtained from all participants and the experimental procedures were approved by the Islamic Azad University, science and research branch, Tehran. Physical and physiological characteristics of participants are showed in Table 1. Inclusion criteria included: healthy adolescent swimmer girls, participated in swimming training 3 - 4 times per week for 3 to 5 years, participants not having any systemic or recurrent disease such as diabetes and asthma, no participants consumed any drugs or supplement during research protocol and they did not have allergy to drugs, Participants had to continue their daily swimming exercise training but they were asked to refrain from intensive exercise in 48 hours before exhausting swimming exercise session.

3.2. Study Design

Three weeks before exhausting exercise session, all participants came to the laboratory for orientation and measurement of maximal oxygen consumption (VO_{2max}). in order to measure VO_{2max}, participants were asked to complete as many laps as possible over a 12-minute test period in an outdoor track. The examiner counted the completed laps over the 12-minute test, while calling out the

Table 1. Participants' Characteristics in Quercetin and Placebo Groups

Variable	Quercetin	Placebo	P Value
Age (y)	15.3 ± 0.17	14.9 ± 0.12	0.324
Weight (kg)	51.3 ± 3.1	52.4 ± 2.6	0.218
Height (cm)	155.4 ± 3.2	154.9 ± 0.5	0.865
BMI ($kg.m^{-2}$)	21.3 ± 2.6	20.8 ± 2.8	0.432
VO_{2max} ($mL\ kg^{-1}\ min^{-1}$)	51.7 ± 4.8	49.6 ± 5.3	0.383
HRmax	193 ± 2	194 ± 2	0.657

time elapsed at 3, 6, and 9 minutes and throughout the test participants were encouraged by administrator. After the 12-minute period, the administrator called participants to stop the test (21). Then, distance covered during the test was determined and Cooper's (22) standardized equation was used in order to convert the covered distance to an estimate of VO_{2max}. All participants were asked to avoid the use of unusual doses of vitamin/mineral supplements (more than 100% of RDA), ergogenic aids and any medications that can affect immune system function during a three-week period before performing the exercise test. However, unfortunately we can't control participants' diet during the study period but they were asked to consume similar and certain amount of foods in the two days before acute exercise session and recommended to set their sleeping time between 11 pm to 7 am. The participants were randomly assigned in two placebo (n = 10) and quercetin (n = 10) groups and blood samples were collected from participations. Then, in double-blind procedures, participants consumed quercetin (1000 mg/day) or placebo supplement for two weeks before exercise test (19). Quercetin supplement was provided as Solaray Quercetin capsules, 500 mg, 90 count. Participants in quercetin group consumed one capsule before their first and last meals of each day (two capsules daily) in order to achieve an intake of 1000 mg quercetin per day. Participants in placebo group received 1 g dextrose daily. participations came to the swimming pool following a two-week period of consuming quercetin or placebo and blood samples collected again after 30 minutes of seated rest as a pre exercise samples. Initially, how to execute the swimming protocol was fully explained to the participants and then exercise protocol was conducted completely by examiner to familiarize participants with protocol and finally swimming exercise session test was performed by all participants. Exercise session consisted of 10 minutes warm up using low intensity crawl swimming followed by crawl swimming with > 85 percent of maximum heart rate until exhaustion. Exercise training protocol consisted of completing one minute bouts of crawl swimming in a 12.5 meter width pool as back and forth swimming that was followed by 30 seconds of inactive rest. These bouts were repeated until participants reached exhaustion. Immediately after exercise, blood samples were collected. Research protocol started during the luteal phase of the participant's menstrual cycle.

3.3. Blood Sample

Blood samples were collected from antecubital vein in supine position. Total WBC and hematologic profiles were measured by using automatic cell counter (Drew Scientific, Excell-22). Remained blood samples were centrifuged at 4°C and 3000 g for 15 minutes. serum samples were stored at -80°C until cytokine analysis.

3.4. Cytokine Analysis and Lactate Measurement

Serum TNF-α levels were measured by enzyme linked immunosorbent assay (ELISA) method (abcam, catalogue: ab46087) according to factory instructions. For measuring lactate levels, Scout lactometer was used and lactate was measured two minutes after exhausting exercise (23).

3.5. Statistical Analysis

Data were analyzed by means of Statistical Package for Social Sciences (version 24.0; SPSS Inc., Chicago, Ill, USA). Data are expressed as means ± SD in Table 1. Data that are expressed in Tables 1 and 2 were analyzed by 2 (groups), 3 (time points) repeated-measures ANOVA that were followed up with a paired sample *t*-test to determine where the difference existed across time within each time.

4. Results

The physical and physiological characteristics of participants and performance data for 20 swimmers that were randomly assigned in two quercetin and placebo groups, are summarized in Table 1. There is no significant difference between two groups at the baseline for physical and physiological characteristics (P > 0.05).

There is no significant difference between baseline and pre-exercise levels of any variables in quercetin and placebo groups (Table 2). According to present study results, a significant interaction effect for TNF-α (P = 0.04), leukocytes (P = 0.001) and monocytes (P = 0.001) was observed in two groups (placebo and quercetin) in different times. In fact, there is significant difference between change in TNF-α, leukocytes and monocytes in placebo and

quercetin groups after an exhausting swimming session ($P < 0.05$).

In addition, levels of TNF-α, lactate and leukocytes significantly increased after exhausting exercise in both quercetin and placebo groups ($P < 0.001$), but significant increase of monocyte levels was only observed in placebo group ($P < 0.001$) (Table 2). According to present study results, there is no significant difference in lactate, neutrophils, lymphocytes, eosinophils and basophil levels in pre to post exercise between quercetin and placebo groups ($P > 0.05$). Leukocytes ($P = 0.07$) and monocytes ($P < 0.001$) increase in placebo group were significant compared to quercetin group after exercise. Also, TNF-α increase in placebo group was significantly more than quercetin group ($P = 0.003$). But, there is no significant difference between two groups in lactate levels after exercise ($P > 0.05$) (Table 2).

5. Discussion

The major result of present study is that two-week quercetin supplementation (1000 mg/daily) resulted in significant decrease in TNF-α response after exhausting swimming compared to placebo group. In contrast to moderate physical activity, long term and high intensity exercise are associated with increasing exercise induced inflammation and oxidative stress (24). Different nutritional compounds have been examined because of their capacity to attenuate immune changes following strenuous exercise and as a result lower the magnitude of physiologic stress. Among these supplementations, quercetin has attracted a lot of attention (18, 25). Quercetin supplementation leads to decrease in circulating pro-inflammatory cytokines, lowering serum cholesterol and phospholipid levels, inhibition of pro-inflammatory pathways and countered oxidative stress (26). In the present study, the inhibitory role of quercetin on inflammatory mediators was confirmed through attenuated TNF-α response following strenuous exercise in quercetin group.

Few studies have examined pre- and post-exercise levels of cytokine following quercetin supplementations. In agreement with our findings, it has been reported that a 24-day period of quercetin supplementations (1000 mg. day) tended to lower plasma levels of IL-8 and TNF-α after 3-hour cycling in trained male cyclists. In addition, quercetin did not attenuate increases in post exercise blood leukocytes (19). These results are in line with the present study findings that indicated significant increase of leukocyte in both quercetin and placebo groups post exercise and this that quercetin isn't associated with decreased leukocyte response to exhausting exercise; even though in the present study we just investigated the effect of quercetin supplementation on TNF-α response following an acute swimming session as a main inflammatory mediator. In another study, MacRae and Mefferd found that quercetin supplementation for six weeks can improve time trail performance in male cyclists. These authors concluded that performance improvement in quercetin groups probably is related to decreased oxidative stress and inflammatory mediator (IL-6) release from muscle (27). Unfortunately, we didn't measure other pro-inflammatory markers. Also, it has been proved that a mixture of antioxidants containing quercetin in untrained males, can attenuate CRP and IL-6 response to eccentric exercise (28). Moreover, the researcher suggested that long term quercetin supplementations (10 weeks) can decrease circulating levels of inflammatory markers (IL-6 and TNF-α) in women afflicted by type 2 diabetes (29). However, we didn't observe significant change in baseline levels of TNF-α after short term quercetin supplementations (two weeks). Collectively, it is suggested that quercetin is unique because of its ability to suppress TNF-a transcription through inhibiting phosphorylation and activation of c-Jun amino-terminal kinase (JNK)/stress-activated protein kinase (SAPK); and as a result, suppressing activation of the transcription factor AP-1 (30). On the other hand, it has been reported that quercetin and other polyphenols can inhibit some of the key elements in cellular signal transduction pathways that cause suppression of pro-inflammatory mediators, and it seems that anti-inflammatory properties of quercetin and phenolic compounds are exerted partly by down regulation of NF-κB pathway (31).

In contrast to present study results, McAnulty et al. reported that acute resveratrol and quercetin supplementation did not attenuate IL-8 pro-inflammatory cytokine increase after one hour of running with %80 of VO_{2max} in healthy trained male adults (20). In this study, 120 mg resveratrol and 225 mg quercetin were ingested over 6 days and 240 mg resveratrol and 450 mg quercetin consumed on day 7, just prior to exercise by participants. However, dose and duration of quercetin supplementations in our study was more than the McAnulty et al. research. In addition, its reported that quercetin supplementation can't decrease markers of muscle damages and inflammatory cytokine response (IL-6 and CRP) after eccentric acute exercise (32). This difference in aforementioned results with present research are probably related to various

Table 2. Baseline and Pre to Post-Exercise Levels of Variables in Quercetin and Placebo Groups After Exhausting Swimming Exercise

Groups	Baseline	After Two Weeks Supple (Pre Exercise)	Immediate Post-Exercise	Effect Size of Exercise (Relative to Pre Exercise), %	P Value	
					P for Treatment (Quercetin)	P for Time; Interaction Effect
TNF-α (pg/mL)					0.032	< 0.001, 0.04
Quercetin	168.6 ± 3.1	173.2 ± 3.9	207.9 ± 11.8	20.3		
Placebo	155.2 ± 2	160.5 ± 4.2	263.2 ± 22.2	63.9		
Lactate (m.mol/L)					0.26	0.02, 0.842
Quercetin	1.9 ± 1.1	2.3 ± 1.2	9.2 ± 3.2	300		
Placebo	2.6 ± 0.9	2.5 ± 1.4	8.9 ± 3.2	256		
Leukocytes ($\times 10^3$ cell/μL)					0.009	0.048, < 0.001
Quercetin	5.27 ± 0.91	5.77 ± 1.05	6.47 ± 1.01	12.13		
Placebo	6.94 ± 0.91	7.36 ± 1.2	10.01 ± 1.15	36		
Neutrophils ($\times 10^3$ cell/μL)					0.38	0.721, 0.680
Quercetin	3.40 ± 0.85	4.06 ± 1.15	3.22 ± 0.56	-20.6		
Placebo	3.34 ± 0.22	3.46 ± 0.24	3.22 ± 0.56	-6.9		
Lymphocytes ($\times 10^3$ cell/μL)					0.75	0.457, 0.274
Quercetin	1.92 ± 0.48	2.35 ± 0.53	2.18 ± 0.68	-7.2		
Placebo	1.94 ± 0.3	1.91 ± .022	2.47 ± 0.36	29.3		
Monocytes ($\times 10^3$ cell/μL)					0.016	< 0.001, < 0.001
Quercetin	0.38 ± 0.09	0.43 ± 0.11	0.44 ± 0.09	2.3		
Placebo	0.37 ± 0.05	0.37 ± 0.07	0.62 ± 0.11	67.5		
Eosinophils ($\times 10^3$ cell/μL)					0.54	0.937, 0.852
Quercetin	0.14 ± 00013	0.16 ± 0.002	0.17 ± 0.0015	6.2		
Placebo	0.15 ± 0.0013	0.15 ± 0.0028	0.16 ± 0.0015	6.6		
Basophil ($\times 10^3$ cell/μL)					0.14	0.204, 0.365
Quercetin	0.08 ± 0.02	0.11 ± 0.01	0.15 ± 0.03	36		
Placebo	0.10 ± 0.01	0.11 ± 0.02	0.14 ± 0.03	27.2		

doses and period of supplementation, in addition to difference in administrated exercise protocols. In general, it can be proposed that acute ingestions of quercetin can't suppress cytokine response after strenuous exercise. In support of this idea, Konrad et al. (18) indicated that ingestion of 1000 mg quercetin 15 min before 2-hour treadmill runs, can't suppress exercise induced increase in inflammatory markers including IL-1β and TNF-α. But the present and other studies (19) reported that 2 - 3 week periods of quercetin supplementations are associated with attenuated exercise related inflammatory response. Another result of the present study is that quercetin supplementation did not affect lactate levels after exercise compared to placebo group. In agreement with our findings it is suggested that, one-week quercetin supplementation (1000 mg daily) did not havet any effect on lactate levels after exercise test for determining VO_{2max} in untrained male and female participants (33). Therefore, the aforementioned effectiveness mechanism of quercetin supplementation on exercise can not be attributed to suppression of lactate production following strenuous exercise and is largely related to anti-inflammatory properties of quercetin. The anti-inflammatory mechanism of quercetin after exercise not fully understood. However, previous research re-

ported that the anti-inflammatory effect of quercetin is partly related to down-regulation of NFκB without significant changes in JNK signaling pathway (34). In addition, some research attributed the anti-inflammatory effect of quercetin to increased gene expression of Th-1-derived IFN-γ and suppressing Th-2-derived cytokine production such as IL-4 by normal peripheral blood mononuclear cells (35). Unfortunately these signaling pathways were no studied in present research. Furthermore, quercetin supplementation affects other signaling pathways including phosphorylated adenosine monophosphate-activated protein kinase (AMPK) signaling (36). However, the exact mechanism by which quercetin decreases inflammation following exercise is yet to be identified and more studies should be conducted in this regard. The main limitation of the present study is lack of measurement of other inflammatory markers such as IL-6 and specially some of anti-inflammatory mediators including IFN-γ. Moreover, unfortunately we couldn't control the participants' diet and physical activity levels accurately during the study period.

5.1. Conclusions

In conclusion, we found that two-week quercetin supplementation resulted in attenuated post exercise increase in pro-inflammatory cytokines such as TNF-α, but quercetin supplementation can't affect lactate changes after exhausting swimming. This study confirmed the hypothesis that quercetin has anti-inflammatory properties and probably quercetin consumption can be an effective strategy in decreasing exercise related inflammation and its side effects in athletes. However, to determine responsible mechanisms, it is necessary to perform more research. Studying long term adaptation to quercetin supplementation and its effects on exercise related inflammation can be suggested in future studies and it is recommend that changes in inflammatory and anti-inflammatory mediators be studied together to determine the exact mechanism of quercetin action on inflammatory status after exercise session. According to present study results, quercetin ingestion is an effective strategy for attenuating exercise induced inflammation.

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