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The Dietary Combination of Quercetin and Resveratrol Supplementation May Improve Exercise Tolerance in Young Untrained Males by Modulating IL-6 and NGAL Response

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Abstract: The effect of antioxidant supplementation on exercise performance has not been clearly defined in trained or untrained individuals. The purpose of this investigation was to test the effects of quercetin-resveratrol combination on plasma anti-inflammatory response and exercise performance in untrained men. After baseline cycling performance testing, the subjects (n=8) were supplemented daily with 500gr quercetin and 500 gr resveratrol for 21 days. The complete blood counts, creatinine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), interleukin-6 (IL-6), Neutrophil gelatinase-associated lipocalin (NGAL) and exercise performance parameters were evaluated at baseline, after 21 days of supplementation and 15 days after the end of the study. The maximal aerobic power (POmax) was increased (19%) in the post-supplementation period compare to baseline and washout period. In the post-supplementation period, the time trial score (TTscore), maximal and average speed significantly increased in the 10 km time trial cycling test compare to the baseline level. Importantly, in response to supplementation, the serum IL-6 and N-GAL levels were upregulated post-supplementation and these levels returned to baseline levels at the end of the washout period. These results suggest that dieatary combination of quercetin and resveretrol may act as a potential tool for enhancing exercise performance by regulation IL-6, NGAL level in untrained men.

Keywords: NGAL, IL6, Cycling performance, Quercetin, Resveratrol

1. Introduction

Quercetinis a natural polyphenolic flavonoid present in a wide variety of food plants, including red onions, apples, and berries [1]. Resveratrol is also a naturally occuring flavonoid present in certain foods, including grapes and red wine [2] and recent studies have reported that resveratrol and quercetin could have a broad range of health benefits, including anti-inflammatory, cardioprotective, chemopreventative, and neuroprotective effects [3-6]. In rodents, resveratrol supplementation is reported to significantly increase aerobic capacities, resistance to fatigue, exercise time, muscle strength, and muscle fiber oxygen consumption [7-11]; however, these benefits have not yet been demonstrated in humans [12]. One of the mechanisms by which resveratrol exerts its effects is by donating electrons from its phenolic hydroxyl groups, thus it acts as an antioxidant and reduces the effects of reactive oxygen species (ROS) leading possibly to an increase in mitochondrial density [13] and decreasing fatigue in mice [6]. However, these effects have not been clear defined in humans [14-16] although an improvement of 3.9% in maximal oxygen uptake (VO_{2peak}) and a decrease of 13.2% in time to the onset of fatigue has been demonstrated [17]. In contrast, such studies have failed to show an ergogenic effect in athletes [14,18] however, one study in elite cyclist has shown a 3% improvement in cycle time trial performance following 6 weeks of quercetin supplementation [19]. Furthermore, Bigelman et al. have found no significant effects in VO_{2peak} or anaerobic performance in trained soldiers [20].

Interleukin-6 (IL-6) is a commonly measured cytokine in the assessment of inflammation and its production is generated by a number of different cell types, inclusive of cytokines, monocytes and macrophages at the site of inflammation [21]. Interleukin-6 plays a number of pro and anti-inflammatory roles within the body [22]; its pro-inflammatory activity incurs T-cell activation, B-cell differentiation, and the stimulation of acute-phase protein production by the hepatocytes in the liver [23,24]. Besides pro-inflammatory properties, IL-6 also has anti-inflammatory effects, e.g. intestinal epithelial cell proliferation, inhibition of epithelial cell apoptosis [25], which are mostly regulated by classical signalling [26]. IL-6 activates adenosine 5'-monophosphate-activated protein kinase (AMPK) in skeletal muscle by increasing the concentration of cyclic adenosine monophosphte (cAMP), and secondarily the AP:ATP ratio [27]. Thus, substantial elevation of IL-6 concentrations may play role in the mobilisation of fuel stores in restoring energy level. Evidently, the studies have shown that carbohydrate ingestion attenuated plasma IL-6 response, while muscle IL-6 mRNA expression was similar in carbohydrate and placebo intake groups [28,29].

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25kDa protein secreted by neutrophils [30], and is present in human tisues, including kidneys, lungs, stomach, colon [31]. NGAL, also known as Lipocalin 2, siderocalin, 24p3, or uterocalin, is a 25 kDa acute phase inflammatory protein, upregulated via various pro-inflammatory stimuli and produced by numerous cell types [32]. Moreover, in recent years, the NGAL has been evaluated as a biomarker in acute kidney injuries [33], it also has the potential to protect against cellular injury mediated by ROS [34,35]. However, the effect of quercetin-resveratrol combination as antioxidant supplementations on plasma IL-6 and NGAL response on exercise performance has not yet been investigated in human. We therefore hypothesised that the combination of quercetin and resveratrol supplementation may improve the physical performance independent of training effects in untrained males by regulation of anti-inflammatory markers.

2. Methods

2.1. Participants and Procedure

Eight healthy, untrained male volunteers (age 22 ± 3.2, weight 68.7± 3.8 kg, and height 177.8± 3.5 cm) were included in this study. The study was performed in accordance with the principles of the Declaration of Helsinki; the protocol was approved by the School of Medicine Balikesir University Ethics committee and informed written consent was obtained from each participant (Ethic prot. number 2014/61).The subjects declaring intake of vitamins or other dietary supplementation, case history of allergy to antioxidant supplements, history of any chronic disease with possible links to oxidative stress, and current symptoms of any acute disease or injuries were excluded from the study. Subjects were also instructed to avoid foods very high in sugar and fat and any other supplementations. The performance test was performed and blood samples were collected at baseline, post-21 days supplementation, and 15 days after the end of the study (washout period). During the test sessions, the participants performed an incremental test to determine maximal aerobic power (POmax) and 10 km time trial (TT) test on separate days. The samples were analyzed for total blood counts, creatinine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), interleukin-6 (IL-6), Neutrophil gelatinase-associated lipocalin (NGAL) as markers for inflammation and anti-inflammation parameters. During the test sessions, maximal aerobic power (POmax), maximal heart rate (MaxHR), time trial score (TTscore), maximal speed (MaxSpeed), cadance (RPM), were evaluated as exercise performance parameters and were compared to base-line values.

2.2. Supplementation

The resveratrol supplement (SolagarInc, USA) consisted of 500 mg Vegetable Capsules and contained natural trans-resveratrol from *polygonumcuspidatum* for optimal antioxidant support free of sugar, salt, corn, yeast, wheat, soy, gluten, dairy products. Solgar's Quercetin Complex included the important bioflavonoid quercetin in a synergistic formula with Ester-C® Plus ad Bromelain. It contains, vitamin C(as Ester-C® calcium ascorbate, 500 mg), calcium (as Ester-C® calcium ascorbate, 50 mg), Quercetin (500 mg), bromelain (50 mg), citrus bioflavonoid complex (50 mg), rose hips (50 mg), acerola (50 mg). Subjects were instructed to take one pill in the morning one pill in the evening of either resveratrol or quercetin for a total intake of 1000 mg/day.

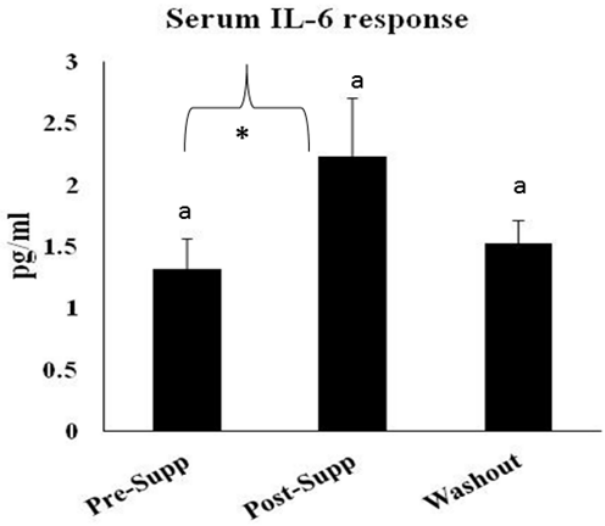

Figure 1. *Effect of quercetin and resveratrol supplementation on IL-6 response*

Values are expressed as mean ± SE (n=8). A significant increase in the post-supplementation period according to baseline and washout $^{a}P<0.05$, analyzed by repeated measures by ANOVA. Significant differences were observed

between Pre-supplementation and post-supplementation period in experimental sessions. *P< 0.05, analyzed by paired-samples t-test. Pre-Supp: Pre-supplementation Post-Supp: Post supplementation.

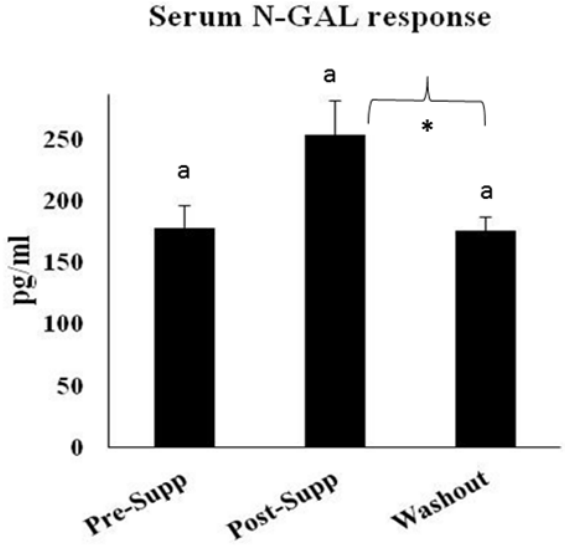

Figure 2. *Effect of quercetin and resveratrol supplementation on N-GAL response*

Values are expressed as mean ± SE (n=8). A significant increase in the post-supplementation period according to baseline and washout $^{a}P<0.05$, analyzed by repeated measures by ANOVA. Significant differences were observed between Post-supplementation and washout period in experimental sessions. *P< 0.05, analyzed by paired-samples t-test. Pre-Supp: Pre-supplementation Post-Supp: Post supplementation.

Table 1. *Effect of supplementation on performance parameters of the participants.*

Maximal incremental test parameters	Pre-supplementation (Mean SE)	Post-supplementation (Mean SE)	Washout (Mean SE)
PO_{max} (W)	224.4 ± 17.4	267.7 ± 19.8	228.9 ± 16.5
HR_{max} (bpm)	188.5 ± 2.7	182.6 ± 3.4	186.6 ± 2.2
Performance parameters during the 10-km cycling time-trial test			
Maximal speed (km/h)	^{a}58.4 ± 2.3**	^{aa}69.1 ± 2.4**	^{aa}55.5 ± 3.4
Average speed (km/h)	^{a}39.2 ± 2.1*	^{a}49.2 ± 2.3*	^{a}37.6 ± 2.6
Final score (min)	^{a}15.7 ± 0.87**	^{a}11.8 ± 0.40**	^{a}13.5 ± 0.38

Values are expressed as mean ± SE (n=8). Significant increase in the post-supplementation period according to baseline and washout $^{a}P<0.05$, $^{aa}P<0.01$, respectively, analyzed by repeated measures by ANOVA. Significant differences were observed between baseline and post-supplementation period in experimental sessions.*P< 0.05,**P< 0.05analyzed by paired-samples t-test.PO_{max}: maximal power output achieved in the incremental test: HR_{max}: maximal heart rate; MaxRPM: maximal revolutions per minute.

Table 2. *Effect of supplementation on haematological, blood chemistry and enzymes parameters of the participants.*

	Pre-supplementation (Mean SE)	***Post-supplementation (Mean SE)***	***Washout (Mean SE)***
WBC $10^3/\mu L$	6.1 ± 0.3	7 ± 0.2	6.2 ± 0.5
RBC$10^3/\mu L$	4.9 ± 0.1	5 ± 0.1	4.9 ± 0.1
HGB (g/dL)	14.4 ± 1	14.8 ± 1.1	14.9 ± 1.1
HCT (%)	43.1 ± 2.9	43.9 ± 1.1	43.7 ± 1.4
Blood chemistry (mg/dl)			
Ca	10 ± 0.1	8.63 ± 1.4	10.3 ± 0.1
Glucose	85.6 ± 4.7	82.7 ± 5.2	92.6 ± 2.2
Pi	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.1
Mg	2 ± 0	2 ± 0.1	2.1 ± 0.1
Enzymes (IU/dL)			
CK	134.1 ± 18.4	149.3 ± 40.9	129.4 ± 33.5
AST	20.1 ± 1.7	21.8 ± 1.2	19.8 ± 1.4
LDH	159.5 ± 11.08	176.8 ± 10.3	149.8 ± 8.9
CRP	0.45 ± 0	0.43 ± 0.1	0.48 ± 0

Values are expressed as mean ± SE (n=8). WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; HCT: hemotocrit; Ca: calcium; Mg: magnesium; CK: creatine kinase; Pi: phosphorus. No significant differences were observed in experimental sessions P>0.05.

2.3. Incremental Test

Participants performed a maximal incremental test on acycle Simulator (Tacx Vortex T1960, Tacx, Wassenaar, Netherlands) that included a 5-min warm-up at a PO corresponding to 100 W, followed by increments of 30 W every 3 min until voluntary exhaustion as judged by the participants not being able to maintain the pedal frequency between 80-90 revolutions (rpm) was reached. Before the each test, the cycle ergotrainer was calibrated in accordance with the manufacturer's recommendations.The POmax was determined as the highest PO that was maintained during the last completed stage. Heart rate was also recorded during the test and the Max HR at the end of the test was noted. If the last stage was not completed, the POmax was calculated from the following equation [36].

$$POmax = POlcs + [(t/180*30)]$$

POlcs is the power output in the last complete stage performed, "t" is the time in seconds sustained in the last incomplete stage "180" is the duration of each stage, and "30" is he increment of PO between the stages.

2.4. 10 km Time-Trials

Participants were asked to cycle a distance of 10 km with free RPM, and were instructed to complete this distance in the shortest possible time.The end of test score (min), maximal speed (km/hr), average speed (km/hr), cadance (RPM) were recorded as performance values.

2.5. Biochemical Analysis

Following centrifugation at 825 xg for 10 min, serum was analyzed for CK, ALT, AST and LDH activities by using commercially available kits on a chemistry autoanalyser (Cobas Integra 800; Roche Diagnostics GmbH; Mannheim, Germany). Serum levels of IL-6 and NGAL were determined by enzyme-linked immuno-sorbent assay (ELISA) using commercially available kits (eBioscience, Austria) on a diagnostic instrument (BioTek, ELx 800, U.S.A).

2.6. Haematological Analysis

Blood samples placed into tubes containing K3-EDTA and were subjected to flow-cytometry for red blood cells (RBC) count, haemoglobin (Hb) concentration, haematocrit (HCT) level, white blood cells (WBC) count by using fully automated Blood Cell Counter Gen-S (Beckman Coulter, Coulter Corporation, USA).

2.7. Statistical Analysis

All calculations were performed using SPSS software (SPSSInc, Chicago, Illinois, USA). The repeated measured analysis of variance (ANOVA) was used with pairwise comparisons. Significant differences between pre- and post supplementation within the group was analyzed by using paired-samples t test. Data are expressed as means ± SE and the level of significance was set at $p< 0.05$.

3. Results

The PO_{max} increased by 19% in the post-supplementation period compared to the baseline and washout period, however, this did not reach statistical significance in the incremental test (P= 0.15) (Table 1). Supplementation with resveratrol and quercetin had no effect on Max HR (P=0.20); however, in the post-supplementation period the final score, maximal and average speed significantly increased in the 10 km time trial cycling test (P= 0.006, P=0.010, P=0.010, respectively) compared to baseline levels (Table 1).

An important finding was that in response to supplementation, the serum IL-6 and N-GAL levels were upregulated in the post-supplementation period and these levels returned to baseline values in the washout period (P=0.026, P=0.016) (Figure 1, 2, respectively). Moreover, haematological and biochemical findings were unaffected by the supplementation during the experimental session (Table 2).

4. Discussion

Our primary findings indicate that 3 weeks of dietary quercetin- resveratrol supplementation (1000 mg/day) significantly affected 10 km TT cycling performance by upregulating cycling speed. However, PO_{max} increased 19% in the post-supplementation period compared to baseline values, although this was not statistically significant.These findings are consistent with published values on the ergogenic effect of quercetin supplementation in humans [16-19], although contrary findings have also been reported [20,38,39]. Furthermore, Scholten&Sergeev have found that quercetin supplementation (6 week, 1000 mg/day) did not enhance performance as measured by VO2 peak running economy, heart rate, and rating of perceived exertion but quercetin can reduced oxidative stress as measured by malondialdehyde as a marker of lipid peroxidation [40]. Ganio et al. found that 5 days of quercetin supplementation did not improve VO2max in untrained, sedantary individuals [38]. Cureton et al. also reported that 1000 mg/day of quercetin supplementation had no significant effect on VO_{2peak} or metabolic responses during submaximal cycling [15]. In contrast Davis et al. (2010) reported that VO_{2max} improved by 3.9% after 7 days of quercetin supplementation (1000 mg/day) in untrained subjects [17]. It has also been reported that quercetin supplementation improves the aerobic

performance with increased the mitocondrial biogenesis by activating the transcription of the sirtuin 1 (SIRT 1) and peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 α) in animal skeletal muscle [16]. However, when quercetin was supplemented during exercise, it attenuated exercise-induced mitochondrial biogenesis in skeletal muscle and increased oxidative stress in the rat model study [41]. Moreover, the same researchers have reported that when quercetin is given during periods of exercise it decreases exercise-induced mitochondrial adaptations in the brain by down-modulation of the PGC-1 α and SIRT1 pathway and it also increases the level of oxidative makers [42]. Accordingly, the quercetin supplementation provides a disadvantage for adaptive response to exercise-induced stress [43]. Evidently, our findings provide additional information that the dietary combination of quercetin and resveratrol supplementation improves the exercise tolerance in untrained humans without training effects and may enhance mitocondrial biogenesis. It is possible that the dietary antioxidant combination of quercetin and resveratol may provide an advantage for adaptive exercise performance in untrained subjects.

In this study, we found that 3 weeks of supplementation upregulated serum IL-6 and NGAL response without affecting inflammatory biomarkes namely, CK, LDH, AST, CRP or blood chemistry levels in humans in the absence of exercise. As far as we are aware this finding has not been reported before and IL-6 is a cytokine and acts on a variety of tissues, and exhibits both pro-and anti-inflammatory properties [44]. The plasma level of IL-6 in healthy humans is typically less than 5 pg mL^{-1}[45]. In our study, the plasma level of the IL-6 indicates a physiological level, although the level IL-6 was upregulated in the post-supplementation period. It has been well documented that the exercise induces plasma IL-6 levels which exerts inflammatory properties [46] and also plays an anti-inflammatory roles within the body [22]. NGAL is a new inflammatory biomarker and was the first reported by Junglee et al (2013) who demonstrated that NGALis upregulated in the heart during exercise [47]. On the other hand one study did not detect any changes in NGAL reponse during eccentric exercise induced muscle damaged when compared the pre-exercise levels [48].The IL-6 response during exercise may improve energy supplies by providing stable blood glucose levels during exercise by upregulating IL-6, however the level of IL-6 in relation to carbohydrate turnover could not be confirmed [49]. Sarvas et al. have found a new insight into the role of the IL-6 in metabolism and energy storage, and they highlight tissue-specific changes in early signaling pathways in response to feeding a four week high fat diet (HFD) to mice [50]. These findings suggest that IL-6 has an important role on the beneficial effects of physical activity in HFD-induced glucose intolerance. Moreover, there is evidence that IL-6 is also linked to activation of the AMPK, which stimulates fatty acid oxidation and increases glucose uptake in the skeletal muscle [51], liver and adipose tissue [52]. Moreover, changes in plasma NGAL have ben correlated with altered kidney function and one of the recent in vitro study has demonstrated that the upregulated NGAL may have play a protective role [53].

Taken together, our findings suggest that 3 weeks of the combination of quercetin and resveratrol supplementation upregulates IL-6 and NGAL levels without any training effects, and may enhance exercise performance in untrained individuals. Thus, dietary combination of quercetin and resveretrol may act as a potential tool for enhancing exercise performance by regulation IL-6, NGAL level in untrained individuals.

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