Citrus Flavonoid Supplementation Improves Exercise Performance in Trained Athletes

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Abstract
Previous studies have shown that polyphenol supplementation may be an effective strategy to improve exercise performance, due to their antioxidant character and ability to stimulate NO production. These properties may contribute to exercise performance, yet no conclusive research has been performed in exploring the direct effects of citrus flavonoids on human exercise performance. Therefore, the purpose of this study was to assess whether supplementation of a customized citrus flavonoid (CF) extract for 4 weeks improves cycling time-trial performance in trained male athletes. In a double-blind, randomized, parallel study, 39 healthy, trained males were given a daily dose of 500 mg of a customized citrus flavonoid extract (CF) or a placebo for 4 weeks. Exercise performance was tested by means of a time-trial test on a cycle ergometer, during which participants had to generate as much power as possible for duration of 10 minutes. Absolute power output significantly increased with 14.9 ± 3.9 W after 4 weeks of CF supplementation, corresponding with a 5.0% increase, compared to 3.8 ± 3.2 W (1.3% increase) in placebo (p < 0.05). In addition, oxygen consumption/power ratio significantly decreased in the CF group compared to placebo (p = 0.001), and a trend was found in the change in peak power output in CF (18.2 ± 23.2 W) versus placebo (-28.4 ± 17.6 W; p = 0.116). The current study is the first convincingly report that citrus flavonoid supplementation can improve exercise performance, as shown by a significant increase in power output during the exercise test.

Key words: Hesperetin, power output, antioxidant, time trial, ergometer.

Introduction

Sport nutrition and nutritional supplementation aimed at enhancing exercise performance and recovery from exercise is commonly used by athletes (Burke and Read, 1993; Froiland et al., 2004; Tscholl et al., 2010). For instance, foods high in carbohydrates and protein are consumed in order to replenish glycogen stores and promote muscle anabolism (Reid, 2013). Although exercise is known to be beneficial for improving exercise performance and preventing several pathological conditions, like cardiovascular disease, type II diabetes, metabolic syndrome, and neurodegenerative diseases, it also induces reactive oxygen species (ROS) production, which is related with several conditions leading to diseases (Davis et al., 2010; Davis et al., 2009; Halliwell, 1991; Malaguti et al., 2013; Masella et al., 2005). Exhaustive exercise increases ROS production, leading to muscle fiber damage, which eventually results in muscle fatigue (Peternelj and Coombes, 2011). However, there is growing evidence suggesting that the presence of a small stimulus, like low concentration of ROS, is able to express the transcription of major antioxidant genes. Enzymes like superoxide dismutase (SOD) and glutathione are important antioxidant defenses that protect cells from ROS-induced oxidative stress (Masella et al., 2005). Moderate exercise acts as a stimulator of the body’s antioxidant defenses against oxidative damage (Gomez-Cabrera et al., 2008; Powers et al., 2011). The correlation between oxidative damage and muscle fatigue could be an important strategy for nutritional interventions to increase exercise performance. Antioxidant supplementation may be an effective strategy, considering the reactive oxygen species (ROS) scavenging effects that could lead to a reduction in muscle damage caused by prolonged exercise (Myburgh, 2014; Sachdev and Davies, 2008).

Polyphenols, including flavonoids derived primarily from fruits, have been of interest due to their antioxidant and anti-inflammatory effects (Masella et al., 2005). Previous studies showed that polyphenols derived from pomegranates, cherries and blueberries reduced muscle soreness and improved muscle strength after eccentric exercise (Bowtell et al., 2011; McLeay et al., 2012; Trombold et al., 2010; 2011). Next to potential benefits in muscle recovery, flavonoid supplementation has been shown to improve endurance exercise performance in humans (Davis et al., 2009; 2010; MacRae and Mefferd, 2006; Nieman et al., 2010).

Another point of interest is the association that has been made with stimulation of nitric oxide (NO) production and subsequent improved endothelial function with different flavonoids, including hesperidin (Rizza et al., 2011). NO acts on smooth muscle cells within the arterial wall, causing vasodilatation and thereby improving blood flow and reducing blood pressure (Cooke et al., 1997; Umans and Levi, 1995). During exercise, this allows for an increase in transport of oxygen and nutrients to the muscles and removes metabolites causing fatigue in prolonged exercise (Bescós et al., 2012; Bloomer, 2010; Wylie et al., 2013).

However, no conclusive clinical research has been performed to explore direct benefits of citrus flavonoids on exercise performance in trained athletes (Malaguti et al., 2013). Therefore, the aim of the study was to elucidate whether a customized citrus flavonoid (CF) extract sup-
plementation for duration of 4 weeks improves cycling
time-trial performance in trained male athletes. It was
hypothesized that CF extract supplementation will im-
prove time-trial performance in trained athletes.

Methods

Participants
Trained, non-smoking male participants, aged 18 – 25 y,
engaging in moderate to high physical activity for a min-
imum of 30 minutes at least 3 times a week were included
in the study. Exclusion criteria were the use of dietary
antioxidant and/or vitamin supplements, and the ingestion
of products containing citrus flavonoids or its metabolite
4 days prior to and during the study.

All participants gave written informed consent be-
fore participation. The study was approved by the Medi-
cal Ethics Committee of Wageningen University and
conducted in full accordance with the principles of the
Declaration of Helsinki of 1975 as amended in 2013 and
with the Dutch Regulations on Medical Research involv-
ing Human Subjects (WMO, 1998). The study was per-
formed at InnoSportlab Papendal, Arnhem, The Nether-
lands (now part of the National Sportcenter Papendal).
The trial has been registered in the Clinical Trials register
(NCT02787733).

Design and protocol
The study was designed as a randomized, parallel group,
double-blind design to test the effects of a daily dose of
500 mg CF extract supplementation versus a placebo over
a period of 4 weeks. A publicly available randomization
program (http://randomizer.org) was used to randomly
assign participants to one of the two interventions.

The study consisted of a familiarization test, a
baseline test (Test 1) and a final test after a 4-week inter-
vention period (Test 2). Prior to each test, participants
were instructed to standardize their workouts and dietary
intake and to refrain from physical exercise and alcohol
for at least 24-h prior to testing. During the complete
duration of the study, participants were instructed to re-
frain from eating foods containing citrus flavonoids, in-
cluding lemons, oranges, and grapefruit.

The familiarization test consisted of a 5 min warm-
up at 100 W followed by a 10 min time-trial on a cycle
ergometer (SRM, Jülich, Germany), during which partici-
pants had to generate the maximal power (W) possible
over the time course of 10 min. Power output (W) and
heart rate (bpm) were measured continuously during the
time-trial, and indirect calorimetry was used to measure
oxygen consumption (VO\textsubscript{2}; mL·min\textsuperscript{-1}) during the time-
trial. In addition, an estimation of maximal oxygen con-
sumption (VO\textsubscript{2max}; mL·min\textsuperscript{-1}) was made. At t = 0, 9, 10
and 11 min after starting the test, participants were asked
to indicate their perceived exhaustion (RPE) using a Borg
scale from 6 – 20, in which 6 is no exertion at all and 20
is maximal exertion. The mean power that was measured
during the familiarization test was used to determine the
power for a 10 min pre-exhaustion test during Test 1 and
Test 2.

The following test protocol was performed identi-
cally on both test days. First, participants had to perform a
standardized pre-exhaustion test, consisting of 10 min
cycling at 80% of the mean work-load as established
during the familiarization test. During this pre-exhaustion
phase, heart rate and exhaustion at t = 0, 9, 10 and 11 min
after start of the test were measured. Subsequently, partic-
ients took a passive rest period of 25 min followed by 5
min of warm-up at 100 W, as a warm-up has been shown
to improve subsequent exercise performance (Fradkin et
al., 2010). Directly after that, participants had to perform
a 10 min time-trial comparable to the familiarization test.
The same parameters were obtained as in the familiariza-
tion test with additional exhaustion measurements at t = -
2, -1 prior to start of the test. Between Test 1 and Test 2,
an intervention period of 4 weeks was conducted, in
which the participants administered either CF extract
supplementation or placebo supplement.

Study product
BioActor BV (Maastricht, the Netherlands) supplied the
CF extract, standardized for hesperetin-7-O-rutinoside 2S
enantiomer, with a total rutinoside content of at least 90% 
(WATTS’UP\textsuperscript{®}/CF extract). The study product was formu-
lated into capsules containing 250 mg of CF extract and
10 mg Magnesium Stearate by Aminolabs (Hasselt, Bel-
gium). The placebo capsules containing 250 mg Micro-
crystalline Cellulose were produced to be identical in
appearance and taste. The capsules were transported and
stored at room temperature. Participants were instructed
to ingest two capsules with 200 mL water daily before
breakfast, for 4 weeks from the first morning after Test 1
(T1) until the morning of Test 2 (T2).

Statistical analysis
Statistical analysis was performed using IBM SPSS Sta-
tistics for Windows (version 22.0 Armonk, NY, USA).
Only data from participants who completed the study (n =
39) were used in the final analysis. Before statistical anal-
ysis, the Shapiro-Wilk test was performed to test for nor-
mality in the test population. To determine the effects of
CF supplementation on the measured performance out-
comes, the mean values at baseline (T1) were compared
with the mean values after 4 weeks of supplementation
(T2). A paired-samples t-test was used to compare the T1
and T2 measurements within both the CF and placebo
group. To compare the differences between the CF and
placebo group, a two-way mixed ANOVA was per-
formed. Data is reported as mean ± standard error of the
mean (SEM) and the level of significance was set on p <
0.05.

Results

Study participants
Thirty-nine participants completed the study protocol, as
one participant dropped out after the first test day due to
an injury not related to the study. The baseline character-
istics of the participants in both groups are shown in Ta-
ble 1. No significant differences were found between CF
extract supplementation and placebo at the start of the 4
week intervention period.
Table 1. Participant characteristics of the 39 participants that completed the study protocol. Data were obtained at baseline and values are expressed as mean (± SEM).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n=39)</th>
<th>Citrus Flavonoid (CF) (n=19)</th>
<th>Placebo (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23.0 (.3)</td>
<td>22.7 (.4)</td>
<td>23.6 (.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.4 (1.29)</td>
<td>74.1 (1.75)</td>
<td>74.8 (1.93)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.84 (.01)</td>
<td>1.83 (.02)</td>
<td>1.84 (.02)</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>22.1 (.30)</td>
<td>22.1 (.42)</td>
<td>22.0 (.44)</td>
</tr>
<tr>
<td>Exercise time per week</td>
<td>9.6 (.6)</td>
<td>8.8 (.6)</td>
<td>10.3 (.9)</td>
</tr>
<tr>
<td>Absolute power (W)</td>
<td>299.3 (7.7)</td>
<td>298.0 (10.8)</td>
<td>300.5 (11.4)</td>
</tr>
<tr>
<td>Relative power (W·kg⁻¹)</td>
<td>4.0 (.1)</td>
<td>4.0 (.2)</td>
<td>4.0 (.2)</td>
</tr>
<tr>
<td>Heart rate (BPM)</td>
<td>174 (2)</td>
<td>171 (4)</td>
<td>177 (3)</td>
</tr>
<tr>
<td>Average VO₂ (mL·kg⁻¹·min)</td>
<td>50.3 (1.3)</td>
<td>51.1 (1.6)</td>
<td>49.6 (2.1)</td>
</tr>
<tr>
<td>Estimated VO₂max (mL·kg⁻¹·min)</td>
<td>57.1 (1.2)</td>
<td>57.9 (1.6)</td>
<td>56.5 (1.8)</td>
</tr>
<tr>
<td>VO₂/Power ratio</td>
<td>.172 (.003)</td>
<td>.176 (.005)</td>
<td>.170 (.004)</td>
</tr>
<tr>
<td>Peak power output (W)</td>
<td>332.5 (21.0)</td>
<td>359.4 (36.0)</td>
<td>306.8 (22.0)</td>
</tr>
</tbody>
</table>

No significant differences were found between CF and placebo group at baseline, p > .05.

Table 2. Within and between group comparison of the performance outcomes of the citrus flavonoid group and placebo group, with corresponding p-values. Values are expressed as mean (± SEM).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Citrus Flavonoid (CF) (n=19)</th>
<th>Placebo (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak power output (W)</td>
<td>332.5 (21.0)</td>
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Exercise performance, power output, and VO₂

Table 2 shows the results of the performance outcomes from the participants with CF extract supplementation and placebo, before and after the 4 week intervention period. Both absolute (p = 0.001) and relative (p = 0.002) power output were significantly increased over the 4 week supplementation in the group receiving CF extract supplementation. No difference in weight was noted during the trial period in either group, making that the relative power parameter in this study did not need to be corrected for changes in weight. In the placebo group, no significant differences in absolute and relative power were found when comparing T2 with T1. The power output of each participant for the whole duration of the time-trial test was also recorded. Figure 1 shows the average power evolution profiles for both groups at baseline and after 4 weeks of CF extract supplementation.

When plotting the mean absolute power output (Figure 2A), it becomes apparent that the group receiving CF extract supplementation significantly increased (p = 0.001) absolute power output by 5% over the 4-week supplementation period, while the placebo group did not (p > 0.05). When expressed as the difference in absolute power output between test days (∆T2 – T1), the increase in power after 4 weeks of CF extract supplementation is significant and almost 4 times higher than placebo (p = 0.032; Figure 2B). In addition, a trend was found for the increase in peak power output (in the first 3 seconds of workout) after 4 weeks of treatment in CF extract supplementation (18.2 ± 23.2 W) versus placebo (-29.0 ± 17.4 W; p = 0.116; Table 2).
Figure 2. A) After 4 weeks of supplementation, the average power in the CF group was increased with 5% ($p = 0.001^{**}$), while no significant increase was noted in the placebo group ($p = 0.243$). Values are expressed as mean ± SEM. B) The increase in power after 4 weeks treatment compared to baseline ($\Delta T2 - T1$) is significantly higher within the CF group than within the placebo group ($p = 0.032^*$). Values are expressed as mean ± SEM.

Figure 3. A) Average VO$_2$ consumption over time in both groups, before and after 4 weeks of intervention. B) The VO$_2$/power ratio was significantly decreased in the CF group compared to the ratio at baseline ($p = 0.001^{**}$). Values are expressed as mean ± SEM.

Discussion

The present study shows that CF extract supplementation for 4 weeks significantly increased absolute power in trained athletes compared to placebo. Furthermore, body mass and VO$_2$ did not change in either groups, resulting in a higher amount of power produced per kilogram of bodyweight (relative power) and per unit of oxygen (VO$_2$/Power ratio) in the CF group compared to placebo.

Previous studies showed that the flavonoid quercetin increased exercise performance and endurance capacity (Davis et al., 2010; MacRae and Mefford, 2006). While in vivo the exact mode of action of flavonoids is unclear, oxygraphic results for quercetin and hesperetin support the shared working mechanism of both compounds by which they can increase exercise performance (Dorta et al., 2005).

In addition to their antioxidant activity, flavonoids may exert other intracellular effects and may interact at the mitochondrial level, contributing to maintain high energetic demand and redox homeostasis. It is suggested that polyphenols may have a potential effect on the functioning of the whole electron transfer chain (ETC), acting on the total mitochondrial respiration process rather than focusing on only a single specific complex (e.g. Complex I) (Santos et al., 1998). In addition, they can act, whether favorably or not, on various mitochondrial processes. Some polyphenols have even been described to act on the signaling pathways (i.e. modulating intrinsic apoptosis (Mertens-Talcott et al., 2003; Zhang et al., 2013)) and trigger mitochondrial biogenesis (i.e. inducing sirtuins) (Baur et al., 2006; Chung et al., 2010; Davis et al., 2009; Sandoval-Acuna, Ferreira and Speisky, 2014a).

The notion that hesperidin behaves in a similar manner as quercetin is supported by several studies that show that quercetin supplementation also increases exercise performance and endurance capacity (Davis et al., 2010; MacRae and Mefford, 2006). In particular, quercetin is also known to increase mitochondrial biogenesis and
Ca²⁺ might stimulate oxidative metabolism by upregulating mitochondrial Ca²⁺ uniporter within the cell, increasing mitochondrial Ca²⁺ concentration. Ca²⁺ regulates several pathways, including one which stimulates eNOS, contributing to an increase of NO production, and stimulating K⁺ efflux (and Ca²⁺ influx), causing hyperpolarization of the cell membrane in endothelial cells (Garland et al., 2011). These results in relaxation of the smooth muscle in blood vessels and with this lowered blood pressure and increased blood flow (Reid, 2013; Umans and Levi, 1995; Wylie et al., 2013). Also, increased mitochondrial Ca²⁺ might stimulate oxidative metabolism by upregulation of pyruvate dehydrogenase and increased of mitochondrial membrane potential, allowing generation of high ATP concentrations lowering oxidative stress and muscle damage, ultimately increasing strength output (Bugger and Abel, 2010; Jouville et al., 1999; Rutter and Rizzuto, 2000). The strong increase in power output in the present study (Table 2; Figure 2) is particularly interesting as it seems in contrast with typical hypotheses that mainly an improved blood flow, and therefore better oxygen delivery to the muscles, can improve exercise performance in athletes (Andersen and Saltin, 1985; Newman et al., 2002). Indeed, the observation that the VO₂max remains unchanged in combination with increased power output supports the potential role of the CF extract in the oxygen maintenance process in the muscle itself, rather than just increased oxygen delivery.

Furthermore, the fact that the positive results were obtained in a study in which well-trained athletes (average 9.6 hours per week) were included, further increases the relevance of the increase in power output. Indeed, it becomes exceedingly more difficult for trained individuals to gain further increase in strength and performance compared to untrained individuals (Ahtiainen et al., 2003; Peterson et al., 2005). An increase of 5% in overall power output is comparable with observed increases after creatine use (Buford et al., 2007). Interestingly, peak power output showed a trend to increase from baseline to 4 weeks after intervention in CF compared to placebo (Table 2). Although not significant (p = 0.112) due to a large variation between participants, a calculated Cohen’s d effect size of 2.70 shows a large magnitude of the treatment in the CF extract supplementation group on peak power output. An increase in peak power output through CF supplementation could be relevant for sports requiring immediate power generation.

To account for potential training adaptation, a familiarization test was conducted before the start of the study. But still, habituation with the test makes the athlete perform better along the study, simply because he is prepared better. Furthermore, daily exercise volume or nature (strength vs. endurance) was not recorded or standardized by participants, although the nature of training has different effect on muscle alterations and metabolism (Baars, 2006). Also, several measurements that would have had added value to the study were not performed to lower the burden for the participant. For example, VO₂max testing could give more insight in aerobic fitness. Or, muscle biopsies would be of interest regarding mitochondrial number and function, for example to determine mitochondrial fractional area, an important quantitative indicator of oxidative capacity. Blood collection prior to and after exercise would have allowed for measurement of multiple blood markers for example related to oxidative stress, such as plasma malondialdehyde, reduced glutathione or cardiac troponin T, a marker for cardiac damage (Bloomer et al., 2005; Rifi et al., 1999). Both blood and muscle biopsies would have allowed a more mechanistic point of view, but were not included in this study due to their invasive character. Nevertheless, the study was placebo-controlled and the heart rate and VO₂ did not change after 4 weeks, making it unlikely that the observed effects were a result of an overall increased fitness or extra effort specifically for the CF extract study group.

Conclusions

Repeated intake of a specific CF extract may improve exercise performance in trained athletes. Therefore, this study is highly relevant for athletes to maximize their performance capacity. The improved performance might be due to improved respiratory efficiency in the mitochondria as a result of CF extract supplementation. However, further mechanistic research is needed to confirm this.

Acknowledgements

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References


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Key points
• Oxygen-processing capacity of mitochondria in the muscles plays a key role in exercise performance and recovery.
• In a double-blind, randomized, parallel study with 39 healthy, trained males the effect of 500 mg of a customized citrus flavonoid extract (CF) on exercise performance was assessed.
• CF intake significantly increased absolute power output with 5% and significantly decreased the oxygen consumption/power ratio, as VO2max remained unchanged.
• The improved performance therefore may be due to improved respiratory efficiency as a result of CF extract supplementation, however, further mechanistic research is needed to confirm this.

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