The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime.

Submitted by: Paola Wollmann Holguín, to the University of Exeter as a dissertation for the degree of Master of Health and Environmental Sciences in Sport and Exercise Science, September 2014.

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I certify that all material in this dissertation which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature:..............................................
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**ABSTRACT**

The purpose of this study was to assess whether 4 weeks of high-intensity low-volume Wingate training consisting of twelve 30s bouts with 2 min recovery periods combined with Montmorency cherry supplementation will provide sufficient stimulus to improve muscle oxidative capacity and performance over and above the effects of HIT training alone. Subjects (n=18) were blindly assigned to placebo group (PG) or treatment group with Montmorency cherry (MCG). Pre and post training measures included: \( \dot{V}O_2 \text{peak} \), end power, gas exchange threshold (GET), lactate threshold and Yo-Yo IR2 distance covered in metres. All groups improved both physiological and performance variables after 4wk training protocol; even though data was not significantly different between groups, there was a strong effect size observed for \( \dot{V}O_2 \text{peak} \), GET and Yo-Yo IR2 distance. MCG increased the distance covered in Yo-Yo IR2 test compared to PG (406.7 ±42.3m and 370 ±20m respectively; with percent change increase of 21.3%, \( d=0.77 \)). Absolute \( \dot{V}O_2 \text{peak} \) increased with MCG supplementation compared to the PG as indicated by the percentage change response across time (5.93% ± 18.72; \( p=.393 \); \( d=0.44 \)). Blood lactate achieved during Ramp incremental exercise increased by 3.05±11.7% compared to the PG 6.04±20.5%; \( p=.22 \). End power at \( \dot{V}O_2 \text{peak} \) showed a percentage change response across time of 11.57±11.28%, \( p=.72 \); \( d=0.30 \). These data indicate that there is a strong tendency towards significance of combining Montmorency cherry supplementation with 4wk HIT regime protocol.

**Key words:** Montmorency cherry supplementation, \( \dot{V}O_2 \text{peak} \), Wingate test, Yo-Yo IR2
INTRODUCTION

Low-volume high-intensity interval training (HIT) has recently been demonstrated to be a time efficient exercise aimed at inducing physiological as well as performance adaptations (25). A specific HIT training protocol called the Wingate test has been widely used in research, with subjects usually performing 4 to 6 (30s all out bouts) inter-spread with 4min rest periods (6,7,8). Unlike endurance training, which relies mainly on oxidative pathways to supply the increased energy demands of the body, HIT produces a fast energy turnover activation of both anaerobic and aerobic pathways (3). Studies by Burgomaster et al. (2008) and Gibala et al. (2006) show that as little as 6 sessions of Wingate training over a 2wk period produce an overall increase in muscle oxidative capacity. Moreover, Gibala et al. (2008), analyzed mitochondrial enzymes and found a 15-35% increase in muscle oxidative capacity after only 6 HIT sessions; in addition they found increases in VO₂peak and glycolytic enzymes. Specifically, HIT produces an increase in glycogen content, a decreased rate of lactate production, an increase in lipid oxidation capacity, increased maximal oxygen uptake (VO₂max) and improvements in exercise performance (2, 8, 25).

Furthermore, recent research by McKay et al. (2009) show that changes in VO₂peak during stepwise exercise testing became much faster after only two HIT training sessions. However the magnitude and direction of these adaptations depend highly on the duration, intensity and frequency of the training protocol as well as the rest periods between bouts. Burgomaster et al. (2006) observed that muscle oxidative capacity was enhanced by only a few brief sessions of HIT. Similarly Bodganis et al. (1996) described a VO₂peak increase from 61% during the first 30s all out bout, to 72% during the second one. Similarly Freese et al. (2013) observed that participants not only had an increase in VO₂max, but that the average work performed decreased followed by a rise in fatigue which was directly proportional to the increased number of bouts performed. Still it remains unknown whether or not the increased trend of VO₂max would continue to increase as the numbers of repeated bouts are increased. However Little et al. (2010) were the first to double the number of intervals per session with participants performing 8 to 12
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intervals in only 6 sessions, which resulted in significant improvements in performance as well as muscle mitochondrial biogenesis. Even though we did not directly assess mitochondrial biogenesis and ROS through enzyme activity we did evaluate if polyphenol supplementation of Montmorency cherry would reduce the oxidative stress produced during high-intensity, low-volume training regime through both performance and physiological measures.

The benefit of a nutritional intervention composed of phytochemicals with antioxidant and anti-inflammatory properties is to decrease the negative effects of extremely high intensity exercise. Polyphenols have been divided into groups based on their chemical structure, with 4 major classes being stilbene, phenolic acids, flavonoids and ligands (Figure 1). Connolly et al. (2006) showed that consuming 45 cherries per day reduce blood markers of inflammation; moreover Jacob et al. (2003) found that cherry juice supplementation decreased strength loss and preserved muscle function after exercise induced muscle damage. Additionally previous studies have shown that polyphenols are known to possess antioxidant properties that scavenge reactive oxygen species ROS, these properties can be directly be measured by assays that determine oxygen radical absorbance capacity (ORAC). Results from the use of these assays show that HIT training decreases ORAC, in contrast polyphenol intake increases ORAC (31, 37). Only a small number of studies have looked at the effects of anthocyanins on exercise performance (5, 11, 56, 59) with poor results showing that 3 weeks of quercetin supplementation did not affect running performance. Similarly MacRae and Mefferd (2006) found a 3.1% improvement in performance after 6wks of quercetin supplementation, but there was not a significant difference compared to the placebo group. Even though the findings are debatable of indicating the efficacy of HIT protocol and polyphenol supplementation, there is a trend towards generating improvements in oxidative capacity, oxidative ATP production and exercise performance.
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Figure 1 Polyphenol Classification: Chemical structure and classification of polyphenols.

Previous research has shown that tolerance to HIT is a function of $\dot{V}O_{2\text{max}}$. The use of dietary supplementation such as polyphenols which alter oxidative pathways may enhance exercise tolerance and performance adaptations. There are over 8,000 variations of polyphenols currently known, of which flavonoids, specifically anthocyanins, have been shown to have a high antioxidant capacity, serving to decrease oxidative damage produced during high stress events such as HIT training (29). It is well known that exercise is a stressor that increases production of reactive oxygen species (ROS) (4). Moreover, altering the balance of oxidative to anti-oxidative ratio leads to altered cell performance and function, yielding an overall decrease in physical performance and fatigue during exercise (31, 33, 37, 40, and 41). It has been demonstrated that mitochondrial ATP production is not capable of supplying the large demands of muscle during HIT training, creating a large accumulation of ROS (8). In addition the higher ROS production exceeds the buffering capacity of antioxidants, resulting as a final process that leads to decreased force production and acute fatigue (19, 30). Since the endogenous mechanisms fail to remove ROS, use of antioxidant supplementation should prevent the negative effects observed during HIT training alone. Therefore the aim of this study was to determine whether adding Montmorency cherry supplementation to a HIT training regime would enhance physiological
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime responses and performance adaptations compared to HIT training in isolation. We hypothesized that a 4wk high-intensity low-volume training protocol combined with Montmorency cherry supplementation will provide sufficient stimulus to improve muscle oxidative capacity and performance over and above the effects of HIT training alone.

METHODS

Subjects

Eighteen healthy young men (age 22.9 ± 3.9 years, height 181.1 ± 6.41 cm, body mass 80.93 ± 12.05 kg, BMI 24.63 ± 3.59 kg.m^{-2}, resting systolic blood pressure (RSBP) 114.17 ± 2.84 mmHg, resting diastolic blood pressure (RDBP) 68.00 ± 9.07 mmHg) volunteered to take part in this study. To take part in the study, subjects had to be recreationally active, aged between 19-35 years, non-hypertensive and non-diabetic, non-smokers, with no previous medical conditions or injuries in the past 6 months. Procedures followed during this study were approved by the Sport and Health Sciences at University of Exeter Research Ethics Committee and conformed to the Declaration of Helsinki. After full disclosure of experimental procedures, all participants were required to give written informed consent. Prior to baseline and post training test days, subjects were asked to hydrate, avoid caffeinated drinks and eat at least 2 hours before arriving to laboratory. A power analysis programme was used to estimate sample size; with alpha set at 0.05 anticipating an interaction effect size of 1.15 and power of 0.80, it was determined that 10 subjects would be needed in each group. However due to the intensity of the training programme as well as the commitment level, 18 participants successfully volunteered and completed the study.

Experimental Protocol

The experimental protocol consisted of familiarization procedures, baseline testing, a 4 week exercise training intervention, and post-training measurements. After assessment of eligibility, using a PAR-Q form, but prior to the commencement of the intervention, participants were randomly allocated to receive a placebo (corn flour) or Montmorency Cherry tablets (CherryActive®, CA). Nutritional information of the dietary supplement shows that each 500mg Montmorency cherry capsule is shown in figure 2.
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<table>
<thead>
<tr>
<th>Analyte</th>
<th>Result</th>
<th>Unit</th>
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<tbody>
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<td>Melatonin</td>
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<td>mcg / g</td>
</tr>
<tr>
<td>Malvidin</td>
<td>6.297</td>
<td>mcg / g</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>47.39</td>
<td>mcg / g</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>1.753</td>
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<td>Peonidin</td>
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<td>Delphinidin</td>
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<td>mcg / g</td>
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<td>Petunidin</td>
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<td>mcg / g</td>
</tr>
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<td>Total Anthocyanins</td>
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<td>mcg / g</td>
</tr>
<tr>
<td>Total Anthocyanosides</td>
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</tr>
<tr>
<td>Total Polyphenols</td>
<td>177.3</td>
<td>mcg / g</td>
</tr>
<tr>
<td>Total Flavonoids</td>
<td>110.6</td>
<td>mcg / g</td>
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</tbody>
</table>

**Figure 2** Montmorency cherry composition: Tart cherry (prunus cerasus) powder analysis performed by Stephen Mohr, director of Quality and Research & Development at Shoreline Fruit, CherryPURE USA.

Subjects were matched and allocated to either the experimental or control group based on the outcome End Power (W) of the Ramp test and Yo-Yo IR2 distance (meters).

Experimental design consisted of a double blind, placebo controlled, randomly assigned, and matched across groups' trial. Participants visited the laboratory on 10 separate occasions. The first visit consisted of anthropometric measurements (height, weight, BMI) and resting blood pressure, then a step incremental-Ramp test was performed. After a 48 hour rest period, participants began their first Wingate training session consisting of 8 repeated 30s all out sprints with 2 minute rest periods in between each sprint, as well as a 24 hour rest period between each training session (described in detail below). During the 4 weeks of Wingate training, subjects were instructed to maintain normal dietary and physical activity practices throughout the study. For 2 days prior to pre and post-training measurement procedures, subjects were asked to refrain from any strenuous exercise and caffeinated drinks, but were encouraged to maintain normal daily activities. After training sessions ended, participants were asked to visit the laboratory one last time for post-training measurements, consisting of anthropometric measurements (height, weight, BMI) as well as resting blood pressure.

**Baseline Testing**

**$\dot{V}O_{2\text{peak}}$ Step-Ramp test**

Subjects performed an incremental test to volitional exhaustion (step and Ramp) on an electronically braked cycle ergometer (Excalibur Sport V2.0, Lode, Groningen, The Netherlands) to determine peak oxygen uptake $\dot{V}O_{2\text{peak}}$ using an automated gas analyser and associated software(Cortex Metalyser 3B, Leipzig, Germany; Metasoft v.2.1 software, Leipzig, Germany). Participants pedalled at their chosen cadence ranging between 70 and 85rpm at unloaded for 3 minutes as warm-up, then the initial load applied was 100W which increased in a stepwise fashion of 30W every 3 minutes
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for 5 stages. After the 5th stage workload was increased linearly by 25W per minute W until the participant was no longer able to maintain a cadence above 65rpm despite vigorous verbal encouragement. Subjects were blinded to the absolute work rate (WR).

Pulmonary gas exchange and ventilation were determined at 10-second averages using Cortex Metalyzer 3B and the highest 15 seconds averaged VO$_2$ at the gas exchange threshold (GET) was identified as the VO$_{2max}$. Additionally, VO$_2$ at the gas exchange threshold (GET) and Ventilation Equivalents were identified as a disproportionate increase in expired VE/V$\text{CO}_2$ (carbon dioxide) relative to VE.\text{V}CO$_2$ (oxygen) (Wasserman et al., 2005). The value used for VO$_{2peak}$ was averaged over the final 30 seconds of the test. The ventilatory threshold and ventilatory slope was determined by visual inspection of plots using criteria described by Caiozzo et al. (1982). Finally, throughout the incremental Ramp test, blood capillary samples were collected from the fingertip 20-s after the beginning of each stage during the step phase. These samples were analysed to determine blood lactate threshold (LT) and glucose (YSI 1500; Yellow Springs Instruments, Yellow Springs, OH).

**Yo-Yo IR2 test**

The Yo-Yo IR2 test consisted of 2 repeated 20 metre runs at a progressively increased speed controlled by audio bleeps. A recording emitted by a CD (www.teknosport.com, Ancona, Italy) marks each initial level with a beep and the subject must run back in time to the initial marker before the second beep is heard. The participants were familiarized with the test protocol on the same day as the testing and were asked to avoid strenuous activity as well as alcohol ingestion 48 hours prior to testing.

The warm up period consisted of the first 3 levels of the Yo-Yo IR2 test as described by Bangsbo et al., (1994); Krustrup et al., (2006). Between each running bout the participants had a 10s resting period before resuming position to start once more. The time between each beep sound is reduced gradually, thus when the participants failed to reach the finishing line in time, the distance covered is recorded and represented the test result. The first time the start marker is not reached a warning is issued, the second time this happens the test would be terminated. For the purpose of the baseline measures for this study, the Yo-Yo IR2 was performed indoor on a 2 metre wide-by-20 meter long running lane marked with cones. Subsequently, the subjects rested for 3 minutes before they performed the Yo-Yo IR2 test to exhaustion. All data collected is expressed as distance (metres covered).
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**Wingate Training**

The specific low-volume high intensity protocol was derived and modified from previous work by (6, 7, 8, 32, 40) Tests were performed using a mechanically braked cycle ergometer (Monark894E peak bike, Monark exercise AB, Sweden). Participants were habituated to the test procedures and protocols during visits 1 and 2. Training commenced 2-3 days after the completion of pre-training measurements. Each participant performed a total of 8 low-volume high-intensity interval training sessions over a 4 week period. The first 4 training sessions consisted of 8 repeated 30-s all out sprints (Wingate anaerobic test), this was progressed to 10 repeated 30-s sprints for the next 2 visits, and then increased to 12 repeated 30-s sprints for the last two sessions. Each HIT session was preceded by a 5 min unloaded warm-up, followed by a 5-s countdown and the bout began applying a load resistance equivalent to 7.5% of their body mass. Subjects were strongly verbally encouraged throughout the bouts. Immediately after the bout ended, participants pedalled with no resistance for 30-s as active recovery rest period, then was asked to fully stop for 90-s between each bout. Participants had at least a 24 hour rest period between training sessions each week.

**Post-Training Measurements**

Approximately 72 h following the final training session, post-training measurements were collected, and consisted of stepwise incremental test-Ramp, anthropometric measurements (height, weight, BMI) and resting blood pressure. After a 48 hour rest period the Yo-Yo IR2 test was conducted this concluded the study.

**Statistical Analysis**

All data are reported as mean and standard deviations unless otherwise stated. Two different statistical models were used for analysis; first all anthropometric data, as well as between group differences were analysed using independent sample t-test (SPSS v19.0, Chicago, IL) resulting in p-values for subsequent inferential analysis. Alpha level of significance was set at p<0.05. Inferential statistics, using 90% confidence intervals (CI) and the effect size (d), were used to derive magnitude based inferences regarding the true value of the observed effect statistic (4). Once assumption of normality was confirmed, parametric tests were performed; a two-way factor ANOVA (2 [cherry or placebo] x 2 [time pre versus post]). When ANOVAs produced a significant difference; those considered to be significant interactions were subsequently analysed using Tukey's post hoc test.
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RESULTS

The MCG supplementation used in this study was well tolerated by participants, with no detrimental side effects. Even though anthropometric variables did not change significantly between groups there was a strong tendency towards significance for BMI, SBP and DBP as seen in table 1.

Table 1 Anthropometric and Physiological variables measured before and after Wingate training (N=18).

<table>
<thead>
<tr>
<th>Placebo + Training (Mean ± SD)</th>
<th>Cherry + Training (Mean ± SD)</th>
<th>%Change</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td><strong>Age, yr</strong></td>
<td>22±4.2</td>
<td>23.8±4.2</td>
<td>-</td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>178.2±6.9</td>
<td>183.7±4.8</td>
<td>-</td>
</tr>
<tr>
<td><strong>Body mass, kg</strong></td>
<td>77.7±8.7</td>
<td>84.1±14.4</td>
<td>83.3±13.4</td>
</tr>
<tr>
<td><strong>BMI (kg·m²)</strong></td>
<td>24.4±2.3</td>
<td>24.9±4.7</td>
<td>24.7±4.4</td>
</tr>
<tr>
<td><strong>Pre ramp resting SBP (mmHg)</strong></td>
<td>110.4±1.3</td>
<td>111.7±7.5</td>
<td>117.9±9.6</td>
</tr>
<tr>
<td><strong>Pre ramp resting DBP(mmHg)</strong></td>
<td>65.3±2.8</td>
<td>63.3±2.4</td>
<td>70.7±9.4</td>
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<tr>
<td><strong>Post ramp SBP(mmHg)</strong></td>
<td>125.7±1.4</td>
<td>115.6±11.4</td>
<td>118.4±17.4</td>
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<tr>
<td><strong>Post ramp DBP(mmHg)</strong></td>
<td>63.7±5.2</td>
<td>62.8±4.4</td>
<td>65.4±5.2</td>
</tr>
</tbody>
</table>

Values are means ± SD (N = 18), except for part of the Yo-Yo IR2 test (N=17) test. Cohen’s d (interaction effect), *Large effect size

Performance variables

2 Yo-Yo IR2 test performance in distance for experimental group: Open bars represent means pre and post training intervention, with error bars showing SE. The solid or dash lines represent individual participant responses.
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All data is expressed as mean ± SD. Distance covered in the Yo-Yo IR2 test before and after the training positively changed by 21.3 ± 20.2 % (406.7m ±42.3 for the MCG, and 370m ±20 for the PG). Even though this difference was not statistically significant (p=0.15) a strong effect size was observed for distance (d=0.77) (Table 2, Figure 2 A, B).

Figure 3 End power during time to exhaustion Ramp cycle test, initiated from a 5 step-wise increase moderate-intensity work rate. (A)Top: end power (W) in MCG pre and post training intervention. (B) end power (W) in PG pre and post training intervention. Open bars represent group means, error bars represent SE, and solid or dash lines represent individual responses to the interventions.

For the MCG, maximal power output achieved during Ramp incremental exercise increased by 10.66± 10.32%, and absolute end power of (320.9W ± 66.7) prior to training to (346.4 W ± 52.6) post 4 week sprint training, but this was not significantly different from the placebo group (p=.58). Similarly End power at VO2peak showed a percentage change response across time of 11.57±11.28%, p=.72; d=0.30), but this difference was not statistically significant between groups (F(1,16)=.312; p=.58 ), these values are summarized in table 3, figure 3A, B.
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**Physiology variables**

![Graph showing VO2peak](image)

Figure 4 VO2peak, pre and post training in MCG compared to PG.

Pulmonary gas exchange threshold (GET) and ventilatory responses to exercise to volitional exhaustion (Ramp) are summarized in table 2, figure 4. Absolute VO2peak was increased with MCG supplementation compared to the PG as indicated by the percentage change response across time (5.93% ± 18.72; p=.393; d=0.44), but these changes were not statistically significant between groups (F(1,16)=.364,p=.55).

![Graph showing GET vslope](image)

![Graph showing GET ventilatory equivalent watts](image)

Figure 5 GET vslope and Figure 6 GET ventilatory equivalents. Open bars and dashed bars represent mean of MCG and PG respectively; error bars represent SE.
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Table 2: Performance and Physiological variables measured before and after Wingate training (N=18)

<table>
<thead>
<tr>
<th></th>
<th>Cherry + Training (Mean ± SD)</th>
<th>Placebo + Training (Mean ± SD)</th>
<th>% Change</th>
<th>d (interaction effect size)</th>
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<tr>
<td><strong>PRE</strong></td>
<td></td>
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<tr>
<td>Absolute VO2peak, L·min⁻¹</td>
<td>3.6±0.68</td>
<td>3.7±0.71</td>
<td>3.9±0.89</td>
<td>4.1±0.6</td>
</tr>
<tr>
<td>Relative VO2peak, ml·kg⁻¹·min⁻¹</td>
<td>47.1±8.7</td>
<td>47.8±8.8</td>
<td>46.3±9.5</td>
<td>48.4±5.5</td>
</tr>
<tr>
<td>Peak power output, W</td>
<td>320.9±66.7</td>
<td>346.4±52.6</td>
<td>323.9±80.1</td>
<td>356.1±63.4</td>
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<tr>
<td><strong>POST</strong></td>
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<tr>
<td></td>
<td>3.7±0.68</td>
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<td>4.1±0.89</td>
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<td></td>
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<td></td>
<td>320.9±66.7</td>
<td>346.4±52.6</td>
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<td><strong>% Change</strong></td>
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<td>11.6±11.</td>
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<td><strong>d</strong> (interaction effect size)</td>
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<td></td>
<td>*Large effect size</td>
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Values are means ± SD (N = 18), except for part of the Yo-Yo IR2 test (N=17) test. d (interaction effect size), *Large effect size

The VO2 at the V-slope anaerobic threshold, the VO2 at the peak work rate, and VO2 at the ventilation equivalent are shown in table 3, figure 5. Percent change over time of the V_slope break point of CO2 production over O2 utilization at the anaerobic threshold was 21% ± 24.3; p=.41; d=0.46), these changes were not associated with a significant effect on the VO2 at GET between groups (F(1,14)=.81, p=.39).

The ventilatory equivalents for O2 (VE/VO2) and CO2 (VE/VCO2) were plotted over time and the level of exercise intensity corresponding to the first rise in VE/VO2 that occurs without a concurrent rise in VE/VCO2 was identified. Percent change over time showed a difference between means (14.53% ± 16.8; p=.52; d=0.33), but this difference was not significant between groups (F(1,16)=0.00, p=.98).

**Lactate Threshold**

For the MCG, blood lactate achieved during Ramp incremental exercise increased by 3.05± 11.7% (320.9 ± 66.7W) compared to the PG, 6.04± 20.5%; however this was not significantly different from the placebo group (p=.22). Furthermore the interaction between the groups and training was not statistically significant (F(1,16)=.07; p=.80 ), these values are summarized in table 3 , figure 3A, B.
DISCUSSION

The main findings in the present study show there was an increase in markers of training status in response to 4 weeks of HIIT. With regards to performance variables, $\dot{V}O_{2\text{peak}}$, end power and Yo-Yo IR2 distance, results showed that there were strong positive relationships between the $\dot{V}O_{2\text{peak}}$ improvements as a result of the 4 weeks of HIIT. This was indicated by a greater time to reach fatigue state during stepwise-Ramp test, higher oxygen uptake and greater distance covered in metres in both MCG and PG. However, the effect was not significantly higher for the supplementation group.

With regards to physiological variables, both LT and GET improved, as observed by a higher end power achieved at the lactate threshold in MCG compared to PG, indicating that there was a higher ATP hydrolysis, however the improvement was not significantly different between groups.

Effect of Low-Volume, High-Intensity Training on Endurance Performance Parameters

Effect of training on $\dot{V}O_{2\text{peak}}$

This study was the first of its kind to use this extreme training regime and increased number of bouts with very little rest in-between in order to evaluate whether physiological adaptations occurred even with a low-volume training time (2 sessions per week of ~20mins). No previous studies had used 12 repeated 30s all out bouts with only 2mins recovery period before. However, the only study that has come close to using a similar protocol was performed by Little et al. (2010), on 7 healthy young males; nevertheless their study had 4min recovery periods and only 6 training sessions over 2 weeks. Based on their results, we had expected to find an increase in $\dot{V}O_{2\text{peak}}$ in the MCG compared to PG. Our results did in fact demonstrate an increased rate of oxygen uptake for both groups. However in contrast to our expectations the mean percent change increase was higher in the PG compared to the MCG (9.8%, 2% respectively), and this effect was not significantly different. Since the increase in absolute $\dot{V}O_{2\text{peak}}$ in the MCG was smaller compared to the PG, a few inferences can be made about why this occurred. Firstly, 3 of the participants in the PG had significantly higher baseline values of $\dot{V}O_{2\text{peak}}$, which possibly be attributed to a higher initial fitness level compared to other individuals in the MCG which in turn might have created a greater response to the training itself. Even though during the blinded matching allocation of subjects we tried to closely group their power outputs as well as fitness levels, it seems from the
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime

data obtained that outliers were produced. Secondly, the increases in $\text{VO}_{2\text{peak}}$ in both groups, regardless of the supplementation indicate that sprint training protocol was enough to induce changes in the ability to generate energy through oxidative metabolism. Gomez-Cabrera et al. (2008) described that moderate intensity training acted as an antioxidant itself by creating bursts of ROS that activate pathways to increase the level of antioxidant enzymes. Additionally Krstrup et al. (2006) and McKenna et al. (1997) observed that the recruitment of type II fast-twitch fibres increased the number of capillaries, thereby increasing the availability for blood flow and oxygen supply to the muscles. Finally, another possibility could be that the participants became more mechanically and chemically efficient during the 4 week training period. This is similar to studies by Aguiló et al. (2003) where performance, measured by $\text{VO}_{2\text{max}}$, increased in both supplementation and placebo group. So the overall response to the training protocol and the effects on $\text{VO}_{2\text{peak}}$ must be attributed as a consequence of initial training status and the intensity of the protocol.

**Effect of training on End Power**

Data from this study shows that there was an increase in end power in MCG pre intervention training compared to post intervention (10.6% change), however this improvement was not significantly greater than the PG (15.6% change). Energy needed for all out bouts lasting more than 10s are derived from anaerobic metabolism (32), while the energy from the recovery time comes from aerobic metabolism (6). A study conducted by Linossier et al. (1993) indicate that short 10s HIT exercises are better for developing anaerobic capacity than longer sprint bouts due to the fact that power decreases exponentially as sprint time increases. Similar to our findings, Stathis et al. (1994) observed that sprint interval training produced an increase in maximal end power and total work performed. Conversely other studies by, Jacobs et al. (2003), as well as Rodas et al. (2000) did not find any significant changes after Wingate training. In our present study the combination of training protocol with Montomorency cherry supplementation resulted in moderate effect size increase in end power in the treatment group compared to the placebo. These findings were similar to those found in a study by Barnett et al. (2004) where six 10s all out bouts interspersed by 60s passive recovery yielded increases in end power during tests to volitional exhaustion. This indicates that our training program may have enhanced the energy provision coming from oxidative metabolism, and that this improvement was higher for the treatment group.

**Effect of training on Yo-Yo IR2 Distance**
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime

The data collected in this study show that mean percent change increased in MCG compared to the PG (27.9% and 16.7% respectively) demonstrating an increase in oxygen uptake (9). The Yo-Yo IR2 test is shown to be sensitive to training adaptations with findings indicating that it can be a useful, cost efficient tool for determining the effects of HIT training protocols on speed endurance design to induce adaptations (40, 41, and 57). Few studies (57, 56) have investigated the effects of acute 4week polyphenol supplementation and the Yo-Yo IR2 test, finding that the improvements in distance covered were due to an increase in buffering capacity of ions and a reduction in intracellular pH. Furthermore, the muscle lactate concentration after completion of the test was higher in the supplementation group. This is similar to studies without supplementation (9) where skeletal muscle lactate accumulation and rate of anaerobic energy turnover was five times larger after completing the Yo-Yo IR2 test, indicating that there is a greater glycogen use by the muscles. Nonetheless, there are limited studies that evaluate the relationship between anaerobic fitness and Yo-Yo IR2 test. Some studies have observed weak or no correlations between the Yo-Yo IR2 test, VO2max and repeated sprint performance (18). On the contrary Krstrup et al. (2003) as well as Thomas et al. (2006) observed a moderate correlation between these variables. Moreover, Krstrup et al. (2006) was able to show a moderate relationship between VO2max and distance covered in the Yo-Yo IR2 test, this is similar to studies by Rampini et al. (2010) where there was a strong correlation between distance and VO2max. In addition, Krstrup et al. (2012) found moderate relationship between peak power produced during Wingate test and Yo-Yo IR2 performance. Similar to the present study where it was found that a strong effect size of relationship between distance and performance during Ramp test, even though it was not a significant relationship, the interaction suggests that repeated HIT yields an elevated rate of anaerobic energy turnover. Although findings from previous research are conflicting in their findings, the positive results suggest that the Yo-Yo IR2 test could be used as an inexpensive predictive test to determine performance adaptations after HIT, and that when combined with Montmorency cherry supplementation the performance adaptations tend to improve over and above the training protocol by itself (18).

Effect of Low-Volume, High-Intensity Training on Endurance Physiology Parameters

Effect of training on Gas Exchange Threshold

Our data demonstrate a mean percentage increase in GET (Vslope power) for both MCG and PG (15.1% and 25.6% respectively); as well as a mean percent increase in
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime

ventilation equivalents for both MCG and PG (11.8% and 17.2% respectively). The effect size of the interaction between Montmorency cherry supplementation and GET was moderate, trending towards significance. This is similar to results observed by McKenna et al. (1997) where the maximal incremental exercise values of VE and VCO2 increased after 7 weeks of sprint training, reflecting an enhanced gas exchange in both lungs and skeletal muscle. Moreover, in the present study the increase in VO2peak and VCO2 during sprint training protocol may have contributed to a decrease in fatigue during the final Ramp test. In contrast, a study conducted in rowers who were supplemented for 6wks with blackcurrant extract did not produce significant differences in physiological parameters, finding only a significantly higher oxygen uptake and lactate concentration. Similarly a quercetin supplementation study found no significant changes between groups in physiological measures; however they did observe an increase in arteriovenous O2 content. The results of our GET measures had a weak effect size, indicating that there were either not enough participants to determine a greater effect, or the supplementation did not alter these parameters.

**Effect of training on Lactate Threshold**

Data from the present study show mean percent change increase of LT in MCG compared to the PG (6.0% and 3.5% respectively). This suggests that lactate accumulation was reduced and therefore glycolysis was able to produce energy at an increased rate to be able to sustain the intense exercise protocol (36). Stone et al. (1987) demonstrated that trained subjects produced higher levels of blood lactate during high intensity training. Therefore the amount of work and time was greater than in the untrained group, indicating that training may prompt better tolerance to lactate accumulation. Similarly studies by Bangsbo et al, (1994) and Dennis et al. (1992) indicate that muscle lactate increased after sprint training was likely due to an increase in muscle perfusion, which in turn created a greater lactate efflux from skeletal muscle to the blood stream in trained athletes compared to middle distance runners. Previous studies have determined that lactate accumulation is related to the ability of producing higher power outputs during SIT protocols (43, 36, and 47). Further to this, Sastre et al. (1992) stated that the ratio between blood lactate and pyruvate is dependent upon the oxidative stress produced during exercise, and in their study using antioxidant supplementation it was observed that LT shifted to the right, meaning that the threshold was attained later in time compared to the placebo group. This also indicates for our present study, that for the MCG the transition between aerobic to anaerobic metabolism was higher than the PG, yielding a higher oxygen consumption (1). Despite the results obtained in this study not eliciting a significant increase in ventilatory
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thresholds, the lactate values reflect that at the anaerobic threshold the subjects in MCG were able to undertake more work compared to the PG as well as use more efficiently oxygen to generate ATP (1, 36). However, in contrast to our findings, Aguiló et al observed that antioxidant supplementation there was a decrease blood lactate concentrations after maximal exercise compared to the placebo group.

Conclusion

The main goal of creating a low-volume high intensity protocol combined with anthocyanins was to improve the capacity of mitochondrial respiration, lactate threshold and physical performance. Despite evidence in past studies showing that supplementation does produce performance adaptations, the present study was unable to determine a significant effect. This could be due to a variety of limitations including small population size, a pre-existing ceiling of mitochondrial content in highly trained participants, whom created outliers. It is also possible that the dose of montmorency cherry given in the study was too high so that may have had negative effects on the antioxidant supplementation and thus may have interfered with mitochondrial biogenesis and thus producing no adaptations over and above the training protocol itself.
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime

REFERENCES

9. Bangsbo
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime


The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime


The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime


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APPENDIX

A. Ethics Approval

Certificate of Ethical Approval

Title: The effects of Montmorency cherry supplementation on adaptations to a high-intensity, low-volume training regime.

Applicants: Paola Wolffmann-Holgain

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: This proposal has been approved until September 2014.

Signature: [Signature]
Date: 15/01/14

Name/Title of Ethics Committee Reviewer: Dr Mark Wilson

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

B. Research Proposal

SPORT AND HEALTH SCIENCES
College of Life and Environmental Sciences
MSc Dissertation Proposal

Deadline: 4pm Friday 10th January 2014
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime

*Please confirm whether the project will be submitted as a Dissertation or a Journal Article. If you are intending to submit in the style of a Journal Article, please confirm which Journal.*

<table>
<thead>
<tr>
<th>Name:</th>
<th>Paola Wollmann</th>
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<tbody>
<tr>
<td>MSc Programme:</td>
<td>Sports and Health Sciences</td>
</tr>
<tr>
<td><strong>SHSM015: Dissertation</strong></td>
<td><strong>YES / NO</strong></td>
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<tr>
<td><strong>SHSM025: Dissertation (Journal Article)</strong></td>
<td><strong>YES / NO</strong></td>
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<tr>
<td><strong>Journal Title:</strong></td>
<td>Does oral Montmorency cherry supplementation enhance the positive cardiovascular adaptations to exercise training in 18-30 year old men by increasing endogenous antioxidant system</td>
</tr>
<tr>
<td><strong>Proposed Title of Dissertation:</strong></td>
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Use the following headings to provide a brief outline of the proposed project
(approx 400 words – this should not exceed 1 page of A4)

1. **Topic of the investigation**
The current study’s aim is to observe and quantify the effects that 6X500mg/ per day of oral Montmorency cherry supplementation for 4 weeks combined with high intensity exercise have on exercise adaptations during oxidative stress challenge in men aged 18-30.

2. **Theoretical framework**
Montmorency cherries are rich sources of polyphenols, high in content of anthocyanins. Current research has focused on unravelling the effects that polyphenolic compound have on decreasing the effects of oxidative stress on muscle cells, furthermore it is believed that ingestion of supplements high in polyphenols during training will increase the physiological effects of exercise on cardiovascular and skeletal systems (Dolinsky et al. 2012).

3. **Procedures and protocols**
Twenty healthy and physically inactive 18-30 year old men will be target population. Inclusion criteria: physical inactivity (<2 hours of moderate intensity/week), no-CVD diagnosis, no co-morbidities and no intake of daily medication. Exclusion criteria includes: <18 or >30 years of age, smokers, pre-existing medical condition, and recreational drug use. Dose of 6X500mg/ per day of Montmorency cherry were calculated through chemical analysis and the manufacturer’s instructions. Randomized blinded placebo controlled design will be conducted, subjects will be allocated to either a combination of high-intensity training program and placebo (n=10) or high-intensity training program plus 6X500mg/day of Montmorency cherry capsules (n=10), based on BMI, Vo2Max and cholesterol levels. Intervention will last for 6 weeks. **Week 1 Pre-testing:** All subjects perform Maximal Incremental Step test using cycle ergometers to record baseline levels of lactate, VO2max, heart rate, and blood pressure. A Yo-YoIR2 test will also be performed to record baseline levels. **Week 2-5 Performance**
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime

**Testing Training Protocol:** Both groups will perform a training session involving 8-12 x 30 second all out cycle spins, with 120 second rest between each bout. This training will be conducted 3 times per week for 4 weeks. The experimental group will be taking Montmorency cherry capsule supplements during this 4-week period, in a dose of 6x500mg/ per day. **Week 6 Post-Intervention Testing:** All subjects will repeat the maximal incremental step test performed during week 1, and subsequently perform a Yo-YoIR2 test. Levels of lactate, VO2max, heart rate, and blood pressure will be measured.

4. **Likely value of results**

   Results will display similar VO2max levels at baseline week 1, as well as compliance to the high-intensity training, with the intensity of the training being equal for both groups (% of training time above 90% of HRmax). There will be a statistically significant reduction observed in blood pressure in experimental group compared to placebo. Anti-inflammatory responses will be statistically significantly greater for experimental group compared to placebo. Final findings will show that there is an additive effect over and above when combined with Montmorency cherry supplementation.

5. **References**


1. **Informed consent for Dissertation**

   Informed consent for dissertations  
   SPORT AND HEALTH SCIENCES  
   College of Life and Environmental Science

**Project title:** The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime.

**Researcher names:** Steve Carter and Paola Wollman
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime

Participant declaration
The purpose of this study has been thoroughly explained to me and any risks involved in my participation have been made explicitly clear. I have had sufficient time to review the information sheet concerning this study and all questions about it have been satisfactorily answered. I understand that I am able to ask further questions at any stage.

In addition, I understand and agree that:

i. Information and data I give will only be used for completion of a dissertation at the College of Life and Environmental Sciences, Sport and Health Sciences at the University of Exeter and publications resulting from the dissertation.

ii. My participation in this study is entirely voluntary and comes with a number of benefits.

iii. I have the right to withdraw any of my data, as well as to withdraw from the study at any time without any disadvantage.

iv. The data will be stored securely to ensure that only the researcher and dissertation supervisor will have access to it.

v. The data will be retained in secure storage until the completion of the dissertation, after which it will be destroyed if it is not used for a publication.

vi. There are some discomforts/risks associated with my participation in the study and I will report immediately to the attending researcher should I experience any discomfort.

vii. The results of the study may be published but my anonymity will be preserved.

After careful consideration regarding my voluntary participation in the above described study, I ______________________________, hereby freely give my informed written consent to my participation in the study.

Participant signature ______________________________ Date ___/___/_____

Witness name (PRINTED) ______________________________ Date ___/___/_____

Witness signature ______________________________
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime
The effects of Montmorency cherry supplementation on performance adaptations to a high-intensity, low-volume training regime