Effects of Montmorency tart cherry (*L. Prunus Cerasus*) consumption on nitric oxide biomarkers and exercise performance.

Keane, KM[1]., Bailey, SJ[2]., Vanhatalo, A.[3], Jones, AM[3]., Howatson, G[1,4].

[1]Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom;

[2]School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom;

[3]Sport and Health Sciences, St. Luke's Campus, University of Exeter, Exeter, United Kingdom;

[4]Water Research Group, School of Environmental Sciences and Development, Northwest University, Potchefstroom, South Africa

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Corresponding Author:

Karen Keane

Department of Sport, Exercise and Rehabilitation

Faculty of Health and Life Sciences

Northumbria University

Newcastle upon Tyne, UK

Tel: 00 44 (0) 191 227 7086

Email: k.keane@northumbria.ac.uk

Abstract

The purpose of this study was to investigate the effects of Montmorency tart cherry juice (MC) on nitric oxide (NO) biomarkers, vascular function and exercise performance in trained cyclists. In a randomized, double blind, placebo (PLA) – controlled, crossover study, 10 trained cyclists (mean ± SD; $\dot{V}O_{2peak}$ 59.0 ± 7.0 ml/kg/min) acutely ingested 30 mL of either MC or PLA and completed a 6 min moderate- and severe-intensity cycling bout 1.5 h post ingestion on two occasions for each experimental condition. The severe-intensity cycling test was continued to exhaustion on one occasion and immediately followed by a 60 s all-out sprint on the other occasion. Blood pressure, pulse wave measures, tissue oxygenation index and plasma [NO_2^-] were assessed pre and 1.5 h post ingestion. Time to exhaustion was not different between conditions ($P > 0.05$), but peak power over the first 20 s (363 ± 42 vs. 330 ± 26 W) and total work completed during the 60 s all-out sprint (21 ± 3 vs. 19 ± 3 kJ) were 10% higher in the MC trial compared to the PLA trial ($P < 0.05$). Systolic blood pressure was 5 ± 2 mmHg lower 1.5 h post MC supplementation compared to PLA supplementation ($P < 0.05$). There were no differences in pulse wave measures, plasma nitrite concentration or tissue oxygenation index between the MC and PLA trials ($P > 0.05$). These results suggest that acute supplementation with MC can lower blood pressure and improve some aspects of exercise performance, specifically end-sprint performance, in trained cyclists.

Keywords: Tart cherries, exercise performance, blood pressure, nitric oxide

AIx	Augmentation index
ANOVA	Analysis of variance
AOX	Antioxidant
BP	Blood pressure
CV	Coefficient of variation
DBP	Diastolic blood pressure
GET	Gas exchange threshold
HbO_2	Oxygenated-haemoglobin
HHb	De-oxygenated-haemoglobin
LSD	Least significant difference
MRT	Mean response time

MC	Montmorency tart cherries
NIRS	Near-infrared spectroscopy
NO	Nitric Oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
eNOS	Endothelial nitric oxide synthase
PLA	Placebo
PWA	Pulse wave analysis
PWV	Pulse wave velocity
SBP	Systolic blood pressure
TOI	Tissue oxygenation index
$\dot{V}CO_2$	CO_2 production
$\dot{V}O_2$	O_2 uptake

Introduction

Montmorency tart cherries (MC) are a rich source of polyphenols [1-3] including the flavonoids isorhamnetin, kaempferol, quercetin, catechin and anthocyanins [4,5]. It has been well documented that these plant compounds are associated with beneficial anti-inflammatory [6], antioxidant [7] (AOX), immunomodulatory and vasodilatory properties [8]. Previous studies demonstrated the positive effects of MC concentrate on indices of cardiovascular function that included increased cell migration [9], cerebral blood flow [10] and reduced systolic blood pressure [10,11]. These effects might be mediated, in part by the ability of polyphenols to facilitate endothelial nitric oxide synthase (eNOS) phosphorylation, thereby increasing endogenous nitric oxide (NO) production [12], however, an increase in NO biomarkers has not been demonstrated with polyphenol-rich MC.

It is possible that the improvements observed in vascular function following MC supplementation may help overcome any potential circulatory limitations that might contribute to exercise fatigue and cessation [13]. Furthermore, increased blood flow has been reported to increase the oxidative energy contribution over the initial stages of exercise and to lower the development of the $\dot{V}O_2$ slow component (a progressive increase in O_2 uptake ($\dot{V}O_2$) as high intensity exercise is continued [14]. Therefore, supplementation with MC might have the potential to improve aspects of the dynamic $\dot{V}O_2$ response during exercise. Consequently, MC might have a positive effect on athletic activities where the rate of blood flow and cardiac output are important determinants of cardiovascular performance, by acting on endothelial function. Further to these mechanisms, quercetin (which is reported to be present in MC [15]) binds and antagonises the adenosine receptor, which could improve performance in a caffeine-like manner [16]. Also, MC concentrate is rich in AOX compounds which may have the ability to augment performance [17].

Despite the potential AOX and vasodilatory properties of tart cherries, to date, only two studies have investigated the effect of tart cherry supplementation on continuous exercise capacity and performance. Clifford and colleagues [18] investigated the influence of different sources of polyphenols on sub-maximal cycling and time trial performance. Supplementation with 200 mg of CherryActive® capsules which contained 216 mg of polyphenols for three days, did not improve cycling time trial performance, heart rate, respiratory exchange ratio, gross mechanical efficiency, oxygen consumption, or blood [lactate] in moderately trained cyclists ($\dot{V}O_{2peak}$ 52.4 ± 8.7 ml/kg/min). In contrast, when participants were supplemented with

CherryPURE® capsules for 10 days, half-marathon completion times were 13% faster and there was a smaller deviation from predicted race pace compared to the placebo trial in trained runners [19], although the mechanism for this improvement was not elucidated. Furthermore, there are several limitations of this study that indicate the results should be interpreted with a degree of caution. Firstly, an independent study design was utilized where participants were matched based on average reported race pace and as a result some variability associated with participant pairing may have been possible. Secondly, differences in aerobic state of training beyond the study inclusion/exclusion criteria may also have been a source of variability in study cohort recruitment. Notwithstanding the limitations of this study, similar performance-enhancing findings have been reported in other studies where polyphenolic content of a fruit-derived supplement is similar to tart cherries [20,21]. Kang and colleagues [20] reported that oligomerized lychee fruit extract increased the anaerobic threshold by 7.4% (1.8, 13.0). Interestingly, these results suggest that a polyphenol-containing supplement and typical AOXs might have different mechanisms of action and that the endurance-promoting effect of oligomerized lychee fruit extract may not directly come from the scavenging of free radicals but might be attributed to other non-AOX properties of polyphenols. More recently, Cook et al. [21] reported that following a seven-day intake of New Zealand blackcurrant extract, there was a significant improvement in cycling time-trial performance by 2.4%, coupled with increased fat oxidation. The authors speculated that this improvement may have been as a result of improved endothelial function and increased peripheral blood flow

Although the potential beneficial role of MC in expediting exercise recovery has been widely demonstrated [22,23], it is still debated as to whether acute MC supplementation can improve endurance exercise performance. Given that the majority of polyphenol compounds are either absorbed or excreted quickly [9,10,24], consequently it makes the argument that a 10-day supplementation period used in a previous study [19] is not necessary to observe improvements in performance in trained individuals. Furthermore, the potential mechanisms that might underpin any ergogenic effects of tart cherry consumption are yet to be fully resolved. Therefore, the purpose of this study was to investigate the effects of acute tart cherry supplementation on plasma NO_2^- concentration ($[NO_2^-]$), a sensitive marker of NOS activity [25], as well as blood pressure, $\dot{V}O_2$ kinetics, muscle oxygenation and exercise performance using a double – blind cross-over experimental study design. We also used near-infrared spectroscopy to provide insight into the matching between skeletal muscle O_2 delivery and utilisation [26] and,

therefore, the potential mechanisms for any improvement in $\dot{V}O_2$ kinetics or exercise performance following acute tart cherry supplementation.

Methods

Participants

Eleven trained male cyclists volunteered to take part in the study, but one participant withdrew after the second study day (mean ± SD age; 28 ± 7 years, stature 1.83 ± 0.06 m, body mass 78.0 ± 8.5 kg and $\dot{V}O_{2peak}$ 59.0 ± 7.0 ml/kg/min). Exclusion criteria for the study were: $\dot{V}O_{2peak}$ < 50 ml/kg/min (determined on visit 1), smoking, food allergy (as discussed with research team), history of gastrointestinal, renal or cardiovascular disease and current use of any food supplementations. All participants provided written, informed consent prior to the commencement of the study. For 24 h prior to and for each of the testing days, participants were asked to avoid strenuous exercise, alcohol, caffeine, nutritional supplements and any anti-inflammatory drugs. Participants were instructed to follow a low phenolic diet for 24 h prior to each arm of the trial by avoiding fruits, vegetables, tea, coffee, alcohol, chocolate, cereals, wholemeal bread, grains and spices and were asked to refrain from strenuous exercise. Compliance with the dietary restrictions was monitored with a standardised, self-reported dietary record. Participants were asked to arrive at the laboratory in a rested and fully hydrated state, ≥10 h postprandial. All tests were performed at the same time of day. The study was conducted in accordance with the Helsinki Declaration and ratified by the University's Research Ethics Committee.

Study Design

Participants were required to report to the laboratory on five occasions over a 4-5 week period to complete the experimental testing (1 familiarization / $\dot{V}O_{2peak}$ visit and 4 experimental visits). On the first visit to the laboratory, participants completed a ramp incremental exercise test for determination of the gas exchange threshold (GET) and peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$). Participants were also familiarized with the two exercise performance tests employed in the study on this visit to avoid any order effect on the performance results as a consequence of a potential "learning effect". Participants then returned to the laboratory on visits 2, 3, 4 and 5 to complete the experimental testing (MC × 2 trials, PLA × 2 trials). During these tests, resting blood pressure, arterial stiffness, pulmonary $\dot{V}O_2$ kinetics during moderate and severe intensity exercise, muscle oxygenation, and exercise performance were assessed, and venous blood samples were obtained. The MC concentrate and placebo (PLA) drinks were administered in a randomized

order as part of a double–blind, crossover experimental design. Each supplementation day was separated by at least 3 days, but no more than 7 days.

Incremental Test.

During the first laboratory visit, participants completed a ramp incremental cycle test on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Initially, participants performed 3 min of baseline cycling at 0 W, after which the work rate was increased by 30 W/min until the limit of tolerance. The participants cycled at a self-selected pedal rate, which, along with saddle and handle bar heights and configuration, was recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas exchange data were collected continuously during the incremental tests and averaged over consecutive 10 s periods. The $\dot{V}O_2$peak was taken as the highest 30 s rolling mean value attained prior to the participant's volitional exhaustion in the test. The GET was determined as 1) the first disproportionate increase in CO_2 production ($\dot{V}CO_2$) from visual inspection of individual plots of $\dot{V}CO_2$ and $\dot{V}O_2$, and an increase in expired ventilation $\dot{V}_E/\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$. The work rate that would require 90% of the GET (moderate – intensity exercise) and 70% Δ (GET + 70% of the difference between the work rate at the GET and $\dot{V}O_2$peak; severe intensity exercise) were calculated. The $\dot{V}O_2$ peak attained in the ramp incremental test was 4.56 ± 0.3 l/min, which equated to a relative $\dot{V}O_2$ peak of 59 ± 7 ml·kg−1·min−1. The work rates that corresponded to 90% GET and 70%Δ were 121 ± 19 and 303 ± 28 W, respectively. The mean response time (MRT) for $\dot{V}O_2$ during ramp exercise was taken into account, specifically two-thirds of the ramp rate was deducted from the work rate at GET and peak $\dot{V}O_2$ (i.e., 20W [27]).

Following the incremental test and a 45-minute rest, participants were familiarized with the exercise tests. Participants completed a moderate- intensity and severe-intensity, step test finishing with an all-out sprint followed (after a 30-minute passive recovery period) by a severe-intensity constant-work-rate step exercise test to the limit of tolerance.

Experimental tests.

On all subsequent visits, participants were required to rest in a seated position for 10 min in an isolated room. Thereafter, baseline blood pressure of the brachial artery was measured using an automated sphygmomanometer (M10-IT Omron Healthcare, UK) according to British Hypertension Society guidelines. Additionally, pulse wave velocity and pulse wave analysis were determined by using Arterial Tonometry (SphygmoCor CPV system, ScanMed Medical, UK). Three measurements were taken, and the mean of the measurements were calculated. A

venous blood sample was then collected into a lithium-heparin tube and centrifuged at 4,000 rpm at 4°C for 10 min, within 2 min of collection. Lithium-heparin plasma was subsequently extracted and immediately frozen at -80°C for later analysis of [NO_2^-] in duplicate via ozone-based chemiluminescence [29].

Participants were then provided with standardised breakfast. Descriptive measures and a Physical Activity Level of 1.7 was used to calculate the participant's individual resting energy expenditure (Schofield Equation, 1985). This subsequently identified the amount of cereal (Rice Snaps, Tesco, Manchester, UK) and semi-skimmed milk (1g/kg/bm) each individual needed to consume to meet 10% of their daily energy requirements. This standardised fixed-energy breakfast meal consisted of a cereal: milk ratio of 30 g: 120 ml and delivered fat, protein and carbohydrate with a macronutrient composition of 14, 14 and 72%, respectively [29]. One-hour post breakfast consumption, participants received the intervention drink. Ninety minutes after ingestion of the supplement, vascular measures were reassessed and participants completed one of the two cycle tests described below, as published pharmacokinetic data have shown that this time frame should coincide with peak plasma concentrations of phenolic acids following MC supplementation [9,11].

The exercise protocol consisted of three "step" exercise tests including two moderate intensity step tests followed by one severe-intensity exercise bout. All participants performed a total of four bouts of moderate intensity exercise and two bouts of severe-intensity exercise for each experimental condition; this protocol replicated previously work [30]. Each transition began with 3 min of baseline cycling at 20 W before an abrupt transition to the target work rate. Each moderate intensity bout lasted 6 min. A passive recovery of 5 min separated the transitions. On two of the study visits (one occasion for each supplement), participants cycled for 6 min at a severe-intensity constant work rate (70% Δ), followed immediately by a 60 s all-out sprint at maximum effort. The resistance on the pedals during this sprint was set using the linear mode of the Lode ergometer, so that each participant would attain the power output calculated to be 50% Δ when considering the participants preferred cadence (linear factor = power/preferred cadence^2). Participants were provided with a 5 s countdown prior to the sprint. On the other two study visits (one occasion for each supplement), the severe-intensity constant-work-rate bout was continued to the limit of tolerance. The time to task failure was used as a measure of exercise tolerance and was immediately recorded when the pedal rate fell by > 10 rpm below the required pedal rate.

Treatments and dietary control

Participants consumed either 60 ml of commercially available MC concentrate (CherryActive®, Hanworth, UK) or fruit-flavoured cordial in a double blind cross-over manner. The choice to use 60 ml was based on previous work that showed a greater uptake of anthocyanin and phenolic acids *in vivo* post-consumption when compared to a 30 ml dose [3,9,11]. The concentrate was diluted with 100 ml of water prior to consumption. The PLA supplement consisted of a commercially available, low fruit (<1%) cordial (Kia Ora, Coca Cola Enterprises, Uxbridge, UK) cordial mixed with water, whey protein isolate (Arla Foods Ltd., Leeds, UK) and maltodextrin (MyProtein Ltd., Northwich, UK), to match the MC concentrate for volume and macronutrient content (Energy = 204 kcal, volume = 60 ml, carbohydrates = 49 g, protein = 2.2 g and fat = 0 g).

Prior to study commencement, it was explained to participants that the aim of the study was to investigate the effect of a fruit juice on vascular function. As a result, they were unaware which beverage was the experimental drink. There were no adverse events reported in response to the intervention products. Subjects consumed all doses of the supplement for each experimental condition, and all participants complied with the low-polyphenolic experimental diet according to the food diaries.

Measurements

During all tests, pulmonary gas exchange and ventilation were measured breath-by-breath. Participants wore a nose clip and breathed through a low-dead-space, low-resistance mouthpiece-and-impeller turbine assembly. Following calibration according to manufacturer's recommendations, the inspired and expired gas volume was continuously sampled at 100 Hz; gas concentration signals were continuously sampled at 100 Hz using paramagnetic (O_2) and infrared (CO_2) analyzers (Oxycon, Care Fusion, Rolle, Switzerland). For data analysis, the moderate bouts of exercise were exported in 10-s averages and then averaged for all bouts. End-exercise $\dot{V}O_2$ (average over the last 30 s and 60 s of the bout), baseline $\dot{V}O_2$ (average over the 60 s prior to exercise) and the amplitude (the difference between the end-exercise and baseline $\dot{V}O_2$) were analysed. For the severe bouts of exercise, the data were exported in 10-s averages and then all bouts were averaged. Baseline $\dot{V}O_2$ (average over the 60 s prior to exercise), the $\dot{V}O_2$ at 120 s (the average from 110 s to 130 i.e. 120 s +/- 10 s) and the end-exercise $\dot{V}O_2$ (the average over the last 30 s of the bout) were identified. The peak $\dot{V}O_2$ was identified using the end- exercise $\dot{V}O_2$. Furthermore, the difference between the baseline and

120 s $\dot{V}O_2$ provides a surrogate for the fundamental amplitude whilst the difference between $\dot{V}O_2$ at 120 s and end-exercise (exhaustion) was used as a surrogate of the $\dot{V}O_2$ slow component.

The oxygenation status of the vastus lateralis of the right leg was monitored near-infrared spectroscopy system (NIRS; INVOS 5100C, Somanetics, Troy, MI, USA) at two different wavelengths (765 nm and 855 nm). The intensity of the transmitted light was continuously recorded at 1 Hz. Based on the absorption and scattering coefficients of light at each wavelength, determined by Beer–Lambert Law, concentrations were estimated for oxy (HbO_2), deoxy (HHb), and total haemoglobin. The leg was initially cleaned around the belly of the muscle, and the optodes were placed 20 cm above the fibular head. The probes were secured to the skin surface and covered with an elasticized, tensor bandage to minimize the influence of extraneous light, and to avoid movement of the probe relative to the skin, while allowing unrestricted movement. The NIRS data were acquired continuously throughout the exercise protocol and output every 5 s and recorded for later offline analysis. The NIRS data output was time stamped at the start of each task segment to assure that data corresponded to the relevant period of task performance. To provide information on muscle oxygenation, NIRS data was averaged at the time points of interest and relative concentration changes in HbO_2 and HHb were calculated.

The tissue oxygenation index (TOI) was calculated using the following equation [30]

$$TOI = \frac{[HbO2]}{[HbO2] + [HHb] \times 100} \quad \text{Equation 1}$$

Pulse wave velocity (PWV) and pulse wave analysis (PWA) were determined by using Arterial Tonometry (SphygmoCor CPV system, ScanMed Medical, UK). The aortic pulse waveform and augmentation index were derived at the radial artery and PWV was determined between carotid and femoral sites. A pencil-type probe was used for all measurements and was held at the base of the neck over the carotid artery and at the inguinal crease over the right femoral artery. Recordings were taken when a reproducible signal was obtained with a high amplitude excursion. The distance between carotid and femoral sites was measured and electrocardiogram gating permitted the time lapse between pulse waves at the carotid and femoral sites to be calculated. Inter- and intra-trial % coefficient of variation (CV) for this method was 3.3 and 3.1%, respectively.

During the exercise trials, a blood sample was collected from a fingertip into a capillary tube at baseline, over the 20 s preceding the step transition in work rate, the 20 s preceding the completion of 360 s of moderate- and severe-intensity cycling exercise, immediately following the 60-s all-out sprint and immediately after exhaustion during the severe-intensity constant-work-rate trial. These whole blood samples were analysed to determine blood lactate (Biosen C_Line, EKF Diagnostic, Barleben, Germany). Intra-sample coefficient of variation for this instrument was 1.8%.

Plasma [nitrate] and [nitrite] determination

All glassware, utensils, and surfaces were rinsed with deionized water to remove residual NO intermediates prior to [NO_2^-] and [NO_3^-] analysis. Plasma samples were deproteinized using zinc sulfate/sodium hydroxide precipitation prior to determination of [NO_3^-]. Firstly, 500 µL of 0.18 N NaOH was added to 100 µL of sample followed by 5 min incubation at room temperature. Subsequently, samples were treated with 300 µL aqueous Z_nSO_4 (5% w/v) and vortexed for 30 s before undergoing an additional 10 min incubation period at room temperature. Samples were then centrifuged at 4,000 rpm for 5 min, and the supernatant was removed for subsequent analysis. The [NO_3^-] of the deproteinized plasma sample was determined by its reduction to NO in the presence of 0.8 % (w/v) VCl_3 in 1 M HCl within an air-tight purging vessel. Plasma samples were introduced to the vessel via 50 µL injections into the septum at the top of the vessel. The spectral emission of electronically excited nitrogen dioxide, derived from the reaction of NO with ozone, was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence nitric oxide analyzer (Sievers NOA 280i. Analytix Ltd, Durham, UK). The [NO_3^-] was determined by plotting signal (mV) area against a calibration plot of sodium nitrate standards. The [NO_2^-] of the undiluted (non-deproteinized) plasma was determined by its reduction to NO in the presence of glacial acetic acid and aqueous NaI (4% w/v) from sodium nitrite standards. 100 µL injections were used for plasma [NO_2^-] determination.

Statistical Analysis

Statistical analysis was performed using PASW Statistics 21.0 for Windows (SPSS, Inc., Chicago, IL.). All group characteristics were reported as means ± standard deviations, unless otherwise stated. A 2 (MC vs. PLA) × 2 (pre vs. post) repeated measures analysis of variance (ANOVA) was employed to assess between–intervention differences in $\dot{V}O_2$, NIRS–TOI, blood pressure, arterial stiffness and lactate. Mauchly's Test of Sphericity was used to check homogeneity of variance for all ANOVA analyses and where necessary, violations of the

assumption were corrected using the Greenhouse–Geisser adjustment. Significant main effects were followed up using LSD *post hoc* analysis. Exercise performance and NO_2- and NO_3- were analysed using a paired samples t-test. Statistical significance was accepted when $P < 0.05$.

Results

Eleven physically active males volunteered to take part in the study, but one participant voluntarily withdrew after the second study day (n=10).

Pulmonary $\dot{V}O_2$ kinetics

The pulmonary $\dot{V}O_2$ data for the moderate- and severe-intensity cycle tests are reported in Table 1. There were no significant between-supplement differences for the baseline and end-exercise $\dot{V}O_2$ during the moderate-intensity step exercise tests ($P > 0.05$). Accordingly, the fundamental $\dot{V}O_2$ amplitude was not significantly different between the conditions (0.55 ± 0.09 and 0.60 ± 0.07 l/min with MC concentrate and PLA respectively, $P > 0.05$).

The baseline and end-exercise $\dot{V}O_2$ during severe-intensity exercise were not significantly impacted by the dietary interventions employed in this investigation ($P > 0.05$ for all comparisons). The $\dot{V}O_2$ at exhaustion was not significantly different between experimental conditions and was also not significantly different from the $\dot{V}O_{2peak}$ attained in the ramp incremental test ($P > 0.05$). No significant differences were reported between MC and PLA in $\dot{V}O_2$ amplitudes from baseline to 120 s of exercise. No differences in $\dot{V}O_2$ slow component were observed across the experimental conditions (Table 1). There were no differences in $\dot{V}CO_2$ between the conditions during moderate- or severe-intensity cycle exercise ($P > 0.05$ for all comparisons).

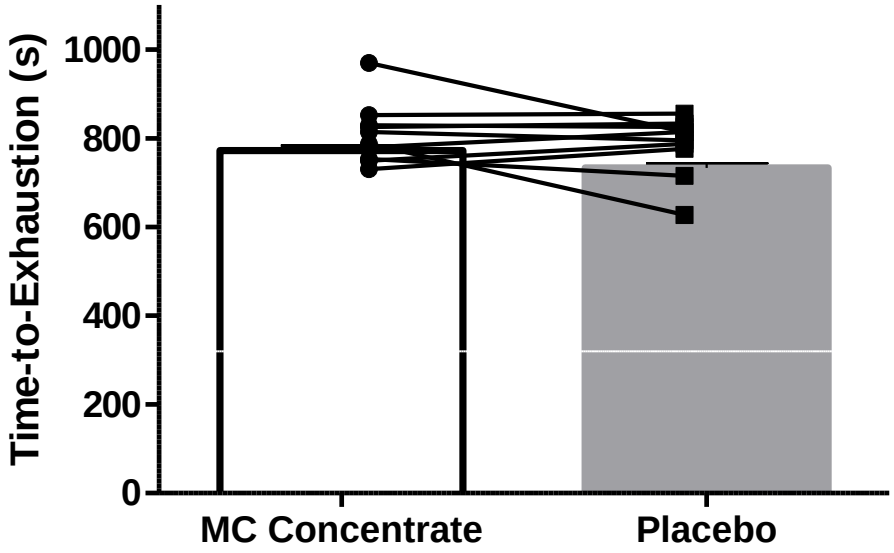
285 Table 1 - Pulmonary $\dot{V}O_2$ measures during moderate- and severe-intensity cycle exercise after
286 MC and PLA supplementation.

	MC Concentrate	Placebo
	Moderate-intensity exercise	
$\dot{V}O_2$, l/min		
Baseline	1.67 ± 0.09	1.68 ± 0.11
End-exercise	2.22 ± 0.09	2.28 ± 0.10
Fundamental amplitude, l/min	0.55 ± 0.09	0.60 ± 0.07
	Severe – intensity exercise	
$\dot{V}O_2$, l/min		
Baseline	1.72 ± 0.04	1.74 ± 0.04
360 s	4.42 ± 0.12	4.36 ± 0.10
Exhaustion	4.50 ± 0.11	4.44 ± 0.09
Fundamental amplitude, l/min	2.43 ± 0.10	2.37 ± 0.09
Slow component amplitude l/min	0.27 ± 0.02	0.35 ± 0.03

287 All values are means ± SEM.
288

289 **Exercise performance**

290 The time to exhaustion during the severe-intensity constant-work-rate cycle trials (the exercise
291 tolerance test) are presented in Fig 1 and while the power profiles for the two experimental
292 conditions during the 60-s all-out sprint that followed the 6-min bout of severe intensity
293 exercise (the exercise performance test) are shown in Fig 2. There were no significant
294 differences in time to exhaustion during the exercise tolerance test between the MC (772 ± 34
295 s) and the PLA conditions (733 ± 34 s, $P = 0.323$). A significant main effect for supplement
296 was observed for the peak power over the first 20 s and total work completed during the 60-s
297 all-out sprint ($P < 0.002$). Follow-up analyses demonstrated that, compared with PLA, MC
298 concentrate supplementation increased the test peak power by 9.5% (363 ± 42 vs. 330 ± 26 W,
299 $P = 0.034$; Fig 2) and the total work completed during the 60 s sprint by 10% between
300 conditions (21 ± 3 vs. 19 ± 3 kJ, $P = 0.021$).



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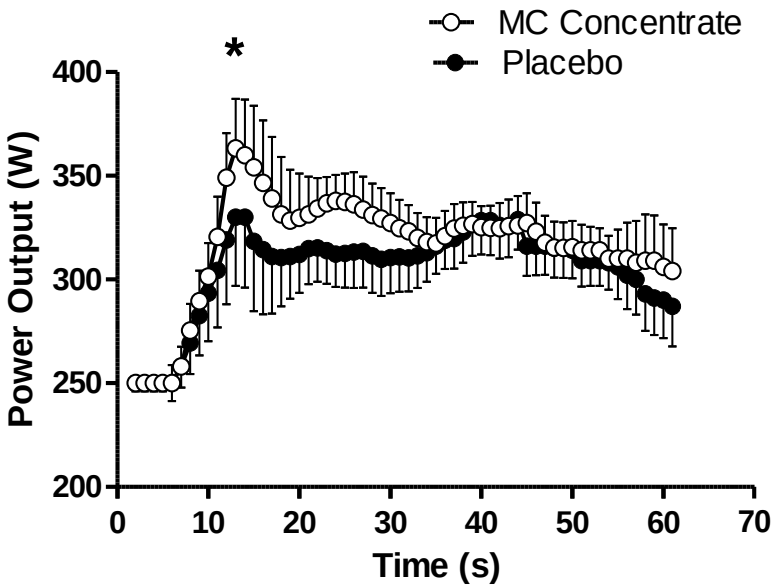
302 Fig 1 - Time to exhaustion during severe-intensity constant –work-rate cycle exercise after MC
303 concentrate and placebo with individual responses to supplementation included. Data presented
304 as means ± SEM.

305



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307 Fig 2 - Group mean power profiles during a 60-s all-out cycle sprint completed immediately
308 after 6-min of severe-intensity cycle exercise following MC concentrate and PLA
309 supplementation. Note significant increase in peak and mean power output during the 60s- all-
310 out sprint after MC concentrate compared to PLA. Data presented as means ± SEM.

311

NIRS

The tissue oxygenation index data during moderate- and severe-intensity cycle exercise with PLA and MC supplementation are reported in Table 2. There were no significant differences between the experimental conditions during the moderate or severe-intensity exercise ($P > 0.05$).

Table 2 - Near – infrared spectroscopy measures during moderate- and severe intensity cycle exercise after MC and PLA supplementation.

	MC Concentrate	**Placebo**
	Moderate- intensity exercise	
Tissue oxygenation index, %		
Baseline	67 ± 2	67 ± 2
120 s	67 ± 2	66 ± 2
End-exercise	66 ± 3	65 ± 1
	Severe-intensity exercise	
Tissue oxygenation index, %		
Baseline	66 ± 3	68 ± 3
120 s	52 ± 4	54 ± 4
End- exercise	49 ± 3	48 ± 5

All values are means ± SEM.

Vascular measures

There was a significant interaction effect for supplement on SBP ($P < 0.05$), with follow-up analyses showing that SBP was lower 1.5 h post MC supplementation, with reductions of 5 ± 2 mmHg compared to the placebo trial. No other vascular variables (DBP, mean arterial pressure (MAP), PWV, augmentation index (AIx) and AIx corrected for HR at 75 bpm) were altered after consumption of the MC concentrate compared to the placebo treatment. The absolute values for all variables are presented in Table 3.

Table 3 - Acute effects of tart Montmorency cherry juice and PLA on vascular function.

Variable	Baseline	1.5 h Post
SBP (mmHg)		
60 ml MC	118 ± 3	115 ± 2*
PLA	119 ± 3	120 ± 3
DBP (mmHg)		
60 ml MC	69 ± 2	68 ± 3
PLA	67 ± 3	68 ± 2
PWV (m/s)		
60 ml MC	6.0 ± 0.3	5.9 ± 0.4
PLA	6.0 ± 0.3	6.0 ± 0.3
MAP (mmHg)		
60 ml MC	85 ± 2	84 ±2
PLA	83 ±2	85 ± 3
AIx (%)		
60 ml MC	9.4 ± 2.0	9.1 ± 1.7
PLA	8.3 ± 2.0	7.5 ± 1.6
AIx @ 75bpm (%)		
60 ml MC	9.2 ± 2.3	9.9 ± 2.2
PLA	9.8 ± 1.8	10.3 ± 2.3

All values are means ± SEM (n=10). * Significant difference between Placebo and cherry concentrate treatment (2-factor repeated measures ANOVA) $P < 0.05$: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PWV, pulse wave velocity; AIx, augmentation index; MC, Montmorency cherry concentrate; PLA, Placebo.

Plasma [NO_2^-] and [NO_3^-]

Due to sampling error, blood was analysed in 8 participants. The plasma [NO_2^-] and [NO_3^-] for the MC and PLA conditions are reported in Table 4. There were no changes for NO_2^- or NO_3^- in the MC supplemented trial when compared to the placebo ($P > 0.05$).

Table 4 - Plasma [NO_2^-] and [NO_3^-] at baseline and 1.5 h following MC concentrate and PLA supplementation.

	MC Concentrate		PLA	
	Pre	**Post**	**Pre**	**Post**
Plasma [NO_2^-] nM	65 ± 9	68 ± 8	63 ± 7	63 ± 9
Plasma [NO_3^-] µM	19 ± 2	18 ± 2	20 ± 2	18 ± 2

All values are means ± SEM (n=8). MC, Montmorency Cherry; PLA, Placebo.

Lactate

There was no treatment or treatment × time interaction effect observed in blood [lactate], however there was a significant time effect identified during both the exercise performance and tolerance test ($P < 0.001$). No other differences were reported. Absolute values are presented in Table 5.

Table 5 - Acute effects of tart MC and PLA on lactate following exercise performance and tolerance test.

	Time points				
	1	**2**	**3**	**4**	**5**
Lactate (mmol/L)	*Exercise performance test*				
60 ml MC	1.7 ± 0.2†	2.3 ± 0.2†	2.6 ± 0.2†	9.5 ± 0.8†	10.6 ± 0.6†
PLA	1.7 ± 0.2†	2.2 ± 0.2†	2.9 ± 0.4†	10.0 ± 0.8†	11.6 ± 0.8†
	Exercise tolerance test				
60 ml MC	2.0 ± 0.1†	2.2 ± 0.2†	2.6 ± 0.2†	9.8 ± 0.7†	12.5 ± 0.8†
PLA	2.1 ± 0.2†	2.4 ± 0.2†	2.7 ± 0.2†	9.9 ± 0.5†	11.2 ± 0.7†

All values are means ± SEM (n=10). † Significant difference between time points from baseline, $P < 0.05$.

Discussion

The principal novel findings from this study are that, compared with an energy-matched placebo, acute MC supplementation enhanced exercise performance, specifically end-sprint performance in trained cyclists, in the absence of changes in $\dot{V}O_2$, plasma [NO_2^-] or muscle oxygenation variables. In addition, SBP was lower 1.5 h post MC consumption but not with PLA.

Influence of MC supplementation on performance

In the current study, peak power output and total work done during a 60-s sprint completed immediately following 6 min of severe-intensity exercise was increased by 9.5 and 10% respectively, following MC relative to the PLA supplementation. While tart cherry supplementation has been shown to improve exercise recovery [22,23], decrease markers of inflammation and oxidative stress [1,3], studies investigating the effects of tart cherries on exercise performance are limited and equivocal. Of the two studies investigating the influence of MC supplementation on exercise performance to date, one reported improved performance in males completing a half marathon (21.1km) run, as evidenced by a faster overall race pace compared to the PLA group [19]. While Levers et al. designed an experiment to assess the influence of ingesting 480 mg of powdered tart cherries for 10-days, including supplementation

on race day up to 48-hr post-run, we investigated the effects of a single dose (60 ml) of MC concentrate on exercise performance using a cross over study design. Despite the differences in dosing strategies, both studies reported improvements in performance in trained participants. Therefore, our findings suggest that acute as well as chronic supplementation with MC concentrate has the potential to improve endurance performance, specially end-sprint performance. Conversely, an earlier study by Clifford and colleagues [18] reported no difference in time trial performance in moderately-trained individuals following the ingestion of 200 mg of powdered tart cherries for 3-days. These conflicting findings are likely linked to the differences in dosing procedures (480mg of providing 991mg of phenolic compounds versus 200mg of providing 216 mg of polyphenols) and exercise performance tests (20 km cycling time trial versus half marathon). There were no differences observed for time to exhaustion between the MC and the PLA trial in the current study. There remains a debate surrounding the applicability and repeatability of the time to exhaustion test [31]. However, a recent addition to the literature highlighted that cycling performance is superior for time to exhaustion versus time trial and therefore should not be disregarded as a useful measure of performance in the laboratory [32].

There were no changes in $\dot{V}O_2$, blood [lactate] or muscle oxygenation in the current study suggesting that the ergogenic effects of MC supplementation were not linked to improved metabolic responses or matching of muscle O_2 supply relative to O_2 demand. Furthermore, plasma [NO_2^-] was not different between the two trials and since plasma [NO_2^-] is a sensitive biomarker of eNOS activity [25], the performance improvements with MC supplementation might be independent of NO-mediated signalling. It is more likely that the enhanced performance might be mediated through the AOX and vasodilatory properties of polyphenol-rich MC. When undertaking high intensity exercise, ROS are produced causing cellular damage and oxidative stress [33]. AOX have the ability to prevent or reduce the extent of oxidative damage to other molecules. It is therefore possible that the AOX effects of MC concentrate were only significant at this time when skeletal muscle contractions were most likely to be compromised by increased oxidative stress [33]. In agreement, an investigation by MacRae and Mefferd [34] reported that the addition of a flavonoid quercetin to a liquid AOX supplement significantly enhanced the AOX effect of the supplement and resulted in a 3.1% performance improvement during a 30 km cycle time trial. Hence, it is possible that a combination of AOX compounds may induce larger effects on exercise performance. Given that MC concentrate has been shown to possess numerous AOX and polyphenolic compounds

[1,2], it makes the argument tenable that the improvement in exercise performance in the current study might be as a result of these AOX compounds. It is worth commenting that MC supplementation could have prolonged the duration for which the participants were in the optimal cellular redox state for force production [35] such that when they were required to produce an all-out sprint, they produced a higher peak power and completed more work. Furthermore, this improvement in exercise performance might also be as a result of an increase in blood flow, as previous research has demonstrated the positive effects of MC supplementation on vascular function, [10,11] that may help to overcome any potential circulatory limitations following strenuous exercise, attenuating the diminishing O_2 supply to the exercising muscles and maintaining force production during the final 60s [36].

Influence of MC supplementation on plasma [NO_2^-]

Nitric oxide is a key regulator of vascular integrity. This multifaceted physiological signalling molecule can be synthesized endogenously through NOS with plasma [NO_2^-] reflecting NOS activity [25]. No significant difference in plasma [NO_2^-] was reported between the MC and PLA trials in the current study. This is somewhat in agreement with the findings from Keane and colleagues [11], where no main effect for plasma nitrate or nitrite was observed following 60 mL MC supplementation using an ELISA kit. Importantly, the lack of a change in plasma [NO_2^-] in the current study extends our previous findings by using a more sensitive method to detect plasma [NO_2^-] in the nM range and this better reflects NOS activity than plasma [NO_3^-] [25]. Since trained endurance cyclists were recruited in the current study, and since endurance training increase NOS expression [37]; it is likely that eNOS-derived NO is already functioning optimally in this cohort and therefore no changes were observed after MC supplementation. It is also interesting to note, the resting plasma NO_2^- levels are quite low in the current study when compared with previous literature [30, 38], this could be as a result of the strict dietary restrictions imposed on the participants on the day preceding the trial or a low intake of nitrate-rich foods in general.

Influence of MC supplementation on blood pressure

A primary outcome of enhanced NO synthesis is a reduction in blood pressure owing to NO-induced smooth muscle relaxation [39]. The current study reported a significant reduction in SBP 1.5 h post MC ingestion relative to placebo, however this augmented modulation occurred in the absence of changes in NO biomarkers. These results are consistent with recent studies demonstrated that supplementation with the NOS substrates, L-Citrulline [30] or L-arginine [38],

lowered blood pressure in the absence of a change in plasma [NO_2^-]. Mechanistically, it would appear that the lowering of BP with acute MC supplementation in the current study is largely NO-independent and is more likely to be a function of the increase in circulating phenolic metabolites post MC ingestion [11].

There was no change in arterial stiffness observed in the current study. This observation is in line with previous studies reporting improved SBP following MC consumption in males with early hypertension [11] and middle aged adults [10], with no improvement in arterial stiffness. It has previously been reported that concurrent improvements in all measures of vascular function are not always observed [40]. Further research is required to investigate the mechanisms by which MC supplementation might positively affect vascular and other physiological responses.

An acknowledged limitation of the current study is the lack of polyphenol analysis and oxidative stress biomarkers. Conceivably, there are a number of mechanisms that could contribute to the physiological effects exerted by MC and as such further research is needed to address the underlying mechanisms of these observations. In addition, participants in the current study abided by strict dietary restrictions in the days preceding the trials and as a result, future work should attempt to investigate the potential synergetic effects of MC supplementation within habitual dietary practices.

In conclusion, this study has shown that acute supplementation with MC juice can lower blood pressure and improve exercise performance, specifically end-sprint performance, in trained endurance cyclists. There were no changes in plasma [NO_2^-], pulmonary $\dot{V}O_2$, or muscle oxygenation after ingesting tart cherry juice so the improvements in blood pressure and exercise performance in this study might be mediated through the potent antioxidant properties of MC juice. The results of this study suggest that supplementation with MC concentrate might represent, a practical, non-pharmacological, dietary intervention to reduce blood pressure and enhance end-sprint performance in trained individuals.

Perspectives

The concept of marginal gains has revolutionised many sports particularly if a sprint finish could potentially be the difference between winning and losing. The improvement in exercise performance in the current study, which supports the supposition of Levers et al. [19], would prove advantageous in sporting situations where very little separates opponents. After completing exercise that was deemed both mechanically and metabolically stressful, when

participants were supplemented with MC, they performed better over a 60-s sprint. Tart cherry juice appears to provide a feasible alternative to pharmaceutical and therapeutic interventions in improving exercise performance. Importantly, regardless of the mechanism, these improvements in performance are of most interest to the athlete, applied coach or sports scientist. Furthermore, the marked reductions in systolic blood pressure highlights the potential importance of MCs as an adjuvant in the management of hypertension, as evidenced previously by Keane and colleagues [10,11].

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