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

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Running head: Montmorency tart cherry consumption and exercise performance

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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 \pm 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 [NO₂⁻] 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

1 **Introduction**

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

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

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

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

53 Although the potential beneficial role of MC in expediting exercise recovery has been widely
54 demonstrated ^{22,23}, it is still debated as to whether acute MC supplementation can improve
55 endurance exercise performance. Given that the majority of polyphenol compounds are either
56 absorbed or excreted quickly ^{9,10,24}, consequently it makes the argument that a 10-day
57 supplementation period used in a previous study ¹⁹ is not necessary to observe improvements
58 in performance in trained individuals. Furthermore, the potential mechanisms that might
59 underpin any ergogenic effects of tart cherry consumption are yet to be fully resolved.
60 Therefore, the purpose of this study was to investigate the effects of acute tart cherry
61 supplementation on plasma NO₂⁻ concentration ([NO₂⁻]), a sensitive marker of NOS activity ²⁵,
62 as well as blood pressure, $\dot{V}O_2$ kinetics, muscle oxygenation and exercise performance using a
63 double – blind cross-over experimental study design. We also used near-infrared spectroscopy
64 to provide insight into the matching between skeletal muscle O₂ delivery and utilisation ²⁶ and,

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

67 **Methods**

68 **Participants**

69 Eleven trained male cyclists volunteered to take part in the study, but one participant withdrew
70 after the second study day (mean \pm SD age; 28 ± 7 years, stature 1.83 ± 0.06 m, body mass
71 78.0 ± 8.5 kg and $\dot{V}O_{2\text{peak}}$ 59.0 ± 7.0 ml/kg/min). Exclusion criteria for the study were: $\dot{V}O_{2\text{peak}}$
72 < 50 ml/kg/min (determined on visit 1), smoking, food allergy (as discussed with research
73 team), history of gastrointestinal, renal or cardiovascular disease and current use of any food
74 supplementations. All participants provided written, informed consent prior to the
75 commencement of the study. For 24 h prior to and for each of the testing days, participants
76 were asked to avoid strenuous exercise, alcohol, caffeine, nutritional supplements and any anti-
77 inflammatory drugs. Participants were instructed to follow a low phenolic diet for 24 h prior to
78 each arm of the trial by avoiding fruits, vegetables, tea, coffee, alcohol, chocolate, cereals,
79 wholemeal bread, grains and spices and were asked to refrain from strenuous exercise.
80 Compliance with the dietary restrictions was monitored with a standardised, self-reported
81 dietary record. Participants were asked to arrive at the laboratory in a rested and fully hydrated
82 state, ≥ 10 h postprandial. All tests were performed at the same time of day. The study was
83 conducted in accordance with the Helsinki Declaration and ratified by the University's
84 Research Ethics Committee.

85 **Study Design**

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

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

99 *Incremental Test.*

100 During the first laboratory visit, participants completed a ramp incremental cycle test on an
101 electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands).
102 Initially, participants performed 3 min of baseline cycling at 0 W, after which the work rate
103 was increased by 30 W/min until the limit of tolerance. The participants cycled at a self-
104 selected pedal rate, which, along with saddle and handle bar heights and configuration, was
105 recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas exchange data
106 were collected continuously during the incremental tests and averaged over consecutive 10 s
107 periods. The $\dot{V}O_{2\text{peak}}$ was taken as the highest 30 s rolling mean value attained prior to the
108 participant's volitional exhaustion in the test. The GET was determined as 1) the first
109 disproportionate increase in $\dot{V}CO_2$ from visual inspection of individual plots
110 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 $\dot{V}_E/\dot{V}CO_2$.
111 The work rate that would require 90% of the GET (moderate – intensity exercise) and 70% Δ
112 (GET + 70% of the difference between the work rate at the GET and $\dot{V}O_{2\text{peak}}$; severe intensity
113 exercise) were calculated. The $\dot{V}O_2$ peak attained in the ramp incremental test was 4.56 ± 0.3
114 l/min, which equated to a relative $\dot{V}O_2$ peak of 59 ± 7 ml·kg⁻¹·min⁻¹. The work rates that
115 corresponded to 90% GET and 70% Δ were 121 ± 19 and 303 ± 28 W, respectively. The mean
116 response time (MRT) for $\dot{V}O_2$ during ramp exercise was taken into account, specifically two-
117 thirds of the ramp rate was deducted from the work rate at GET and peak $\dot{V}O_2$ (i.e., 20W²⁷).

118

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

123 *Experimental tests.*

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

130 venous blood sample was then collected into a lithium-heparin tube and centrifuged at 4,000
131 rpm at 4°C for 10 min, within 2 min of collection. Lithium-heparin plasma was subsequently
132 extracted and immediately frozen at -80°C for later analysis of [NO₂⁻] in duplicate via ozone-
133 based chemiluminescence ²⁹.

134

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

147

148 The exercise protocol consisted of three "step" exercise tests including two moderate intensity
149 step tests followed by one severe-intensity exercise bout. All participants performed a total of
150 four bouts of moderate intensity exercise and two bouts of severe-intensity exercise for each
151 experimental condition; this protocol replicated previously work ³⁰. Each transition began with
152 3 min of baseline cycling at 20 W before an abrupt transition to the target work rate. Each
153 moderate intensity bout lasted 6 min. A passive recovery of 5 min separated the transitions.
154 On two of the study visits (one occasion for each supplement), participants cycled for 6 min at
155 a severe-intensity constant work rate (70% Δ), followed immediately by a 60 s all-out sprint at
156 maximum effort. The resistance on the pedals during this sprint was set using the linear mode
157 of the Lode ergometer, so that each participant would attain the power output calculated to be
158 50% Δ when considering the participants preferred cadence (linear factor = power/preferred
159 cadence²). Participants were provided with a 5 s countdown prior to the sprint. On the other
160 two study visits (one occasion for each supplement), the severe-intensity constant-work-rate
161 bout was continued to the limit of tolerance. The time to task failure was used as a measure of
162 exercise tolerance and was immediately recorded when the pedal rate fell by > 10 rpm below
163 the required pedal rate.

164 **Treatments and dietary control**

165 Participants consumed either 60 ml of commercially available MC concentrate
166 (CherryActive®, Hanworth, UK) or fruit-flavoured cordial in a double blind cross-over manner.
167 The choice to use 60 ml was based on previous work that showed a greater uptake of
168 anthocyanin and phenolic acids *in vivo* post-consumption when compared to a 30 ml dose^{3,9,11}.
169 The concentrate was diluted with 100 ml of water prior to consumption. The PLA supplement
170 consisted of a commercially available, low fruit (<1%) cordial (Kia Ora, Coca Cola Enterprises,
171 Uxbridge, UK) cordial mixed with water, whey protein isolate (Arla Foods Ltd., Leeds, UK)
172 and maltodextrin (MyProtein Ltd., Northwich, UK), to match the MC concentrate for volume
173 and macronutrient content (Energy = 204 kcal, volume = 60 ml, carbohydrates = 49 g, protein
174 = 2.2 g and fat = 0 g).

175

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

182 **Measurements**

183 During all tests, pulmonary gas exchange and ventilation were measured breath-by-breath.
184 Participants wore a nose clip and breathed through a low-dead-space, low-resistance
185 mouthpiece-and-impeller turbine assembly. Following calibration according to manufacturer's
186 recommendations, the inspired and expired gas volume was continuously sampled at 100 Hz;
187 gas concentration signals were continuously sampled at 100 Hz using paramagnetic (O₂) and
188 infrared (CO₂) analyzers (Oxycon, Care Fusion, Rolle, Switzerland). For data analysis, the
189 moderate bouts of exercise were exported in 10-s averages and then averaged for all bouts.
190 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
191 the 60 s prior to exercise) and the amplitude (the difference between the end-exercise and
192 baseline $\dot{V}O_2$) were analysed. For the severe bouts of exercise, the data were exported in 10-s
193 averages and then all bouts were averaged. Baseline $\dot{V}O_2$ (average over the 60 s prior to
194 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-
195 exercise $\dot{V}O_2$ (the average over the last 30 s of the bout) were identified. The peak $\dot{V}O_2$ was
196 identified using the end- exercise $\dot{V}O_2$. Furthermore, the difference between the baseline and

197 120 s $\dot{V}O_2$ provides a surrogate for the fundamental amplitude whilst the difference between
198 $\dot{V}O_2$ at 120 s and end-exercise (exhaustion) was used as a surrogate of the $\dot{V}O_2$ slow component.

199 The oxygenation status of the vastus lateralis of the right leg was monitored near-infrared
200 spectroscopy system (NIRS; INVOS 5100C, Somanetics, Troy, MI, USA) at two different
201 wavelengths (765 nm and 855 nm). The intensity of the transmitted light was continuously
202 recorded at 1 Hz. Based on the absorption and scattering coefficients of light at each
203 wavelength, determined by Beer–Lambert Law, concentrations were estimated for oxy (HbO₂),
204 deoxy (HHb), and total haemoglobin. The leg was initially cleaned around the belly of the
205 muscle, and the optodes were placed 20 cm above the fibular head. The probes were secured
206 to the skin surface and covered with an elasticized, tensor bandage to minimize the influence
207 of extraneous light, and to avoid movement of the probe relative to the skin, while allowing
208 unrestricted movement. The NIRS data were acquired continuously throughout the exercise
209 protocol and output every 5 s and recorded for later offline analysis. The NIRS data output
210 was time stamped at the start of each task segment to assure that data corresponded to the
211 relevant period of task performance. To provide information on muscle oxygenation, NIRS
212 data was averaged at the time points of interest and relative concentration changes in HbO₂ and
213 HHb were calculated.

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

$$215 \text{ TOI} = \frac{[\text{HbO}_2]}{[\text{HbO}_2] + [\text{HHb}] \times 100} \quad \text{Equation 1}$$

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

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

234 **Plasma [nitrate] and [nitrite] determination**

235 All glassware, utensils, and surfaces were rinsed with deionized water to remove residual NO
236 intermediates prior to $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ analysis. Plasma samples were deproteinized using
237 zinc sulfate/sodium hydroxide precipitation prior to determination of $[\text{NO}_3^-]$. Firstly, 500 μL
238 of 0.18 N NaOH was added to 100 μL of sample followed by 5 min incubation at room
239 temperature. Subsequently, samples were treated with 300 μL aqueous ZnSO_4 (5% w/v) and
240 vortexed for 30 s before undergoing an additional 10 min incubation period at room
241 temperature. Samples were then centrifuged at 4,000 rpm for 5 min, and the supernatant was
242 removed for subsequent analysis. The $[\text{NO}_3^-]$ of the deproteinized plasma sample was
243 determined by its reduction to NO in the presence of 0.8 % (w/v) VCl_3 in 1 M HCl within an
244 air-tight purging vessel. Plasma samples were introduced to the vessel via 50 μL injections
245 into the septum at the top of the vessel. The spectral emission of electronically excited nitrogen
246 dioxide, derived from the reaction of NO with ozone, was detected by a thermoelectrically
247 cooled, red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence
248 nitric oxide analyzer (Sievers NOA 280i, Analytix Ltd, Durham, UK). The $[\text{NO}_3^-]$ was
249 determined by plotting signal (mV) area against a calibration plot of sodium nitrate standards.
250 The $[\text{NO}_2^-]$ of the undiluted (non-deproteinized) plasma was determined by its reduction to NO
251 in the presence of glacial acetic acid and aqueous NaI (4% w/v) from sodium nitrite standards.
252 100 μL injections were used for plasma $[\text{NO}_2^-]$ determination.

253 **Statistical Analysis**

254 Statistical analysis was performed using PASW Statistics 21.0 for Windows (SPSS, Inc.,
255 Chicago, IL.). All group characteristics were reported as means \pm standard deviations, unless
256 otherwise stated. A 2 (MC vs. PLA) \times 2 (pre vs. post) repeated measures analysis of variance
257 (ANOVA) was employed to assess between-intervention differences in $\dot{V}\text{O}_2$, NIRS-TOI,
258 blood pressure, arterial stiffness and lactate. Mauchly's Test of Sphericity was used to check
259 homogeneity of variance for all ANOVA analyses and where necessary, violations of the

260 assumption were corrected using the Greenhouse–Geisser adjustment. Significant main effects
261 were followed up using LSD *post hoc* analysis. Exercise performance and NO₂⁻ and NO₃⁻
262 were analysed using a paired samples t-test. Statistical significance was accepted when $P <$
263 0.05.

264 **Results**

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

267 **Pulmonary $\dot{V}O_2$ kinetics**

268 The pulmonary $\dot{V}O_2$ data for the moderate- and severe-intensity cycle tests are reported in Table
269 1. There were no significant between-supplement differences for the baseline and end-exercise
270 $\dot{V}O_2$ during the moderate-intensity step exercise tests ($P > 0.05$). Accordingly, the fundamental
271 $\dot{V}O_2$ amplitude was not significantly different between the conditions (0.55 ± 0.09 and $0.60 \pm$
272 0.07 l/min with MC concentrate and PLA respectively, $P > 0.05$).

273

274 The baseline and end-exercise $\dot{V}O_2$ during severe-intensity exercise were not significantly
275 impacted by the dietary interventions employed in this investigation ($P > 0.05$ for all
276 comparisons). The $\dot{V}O_2$ at exhaustion was not significantly different between experimental
277 conditions and was also not significantly different from the $\dot{V}O_{2peak}$ attained in the ramp
278 incremental test ($P > 0.05$). No significant differences were reported between MC and PLA in
279 $\dot{V}O_2$ amplitudes from baseline to 120 s of exercise. No differences in $\dot{V}O_2$ slow component
280 were observed across the experimental conditions (Table 1). There were no differences in
281 $\dot{V}CO_2$ between the conditions during moderate- or severe-intensity cycle exercise ($P > 0.05$ for
282 all comparisons).

283

284

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

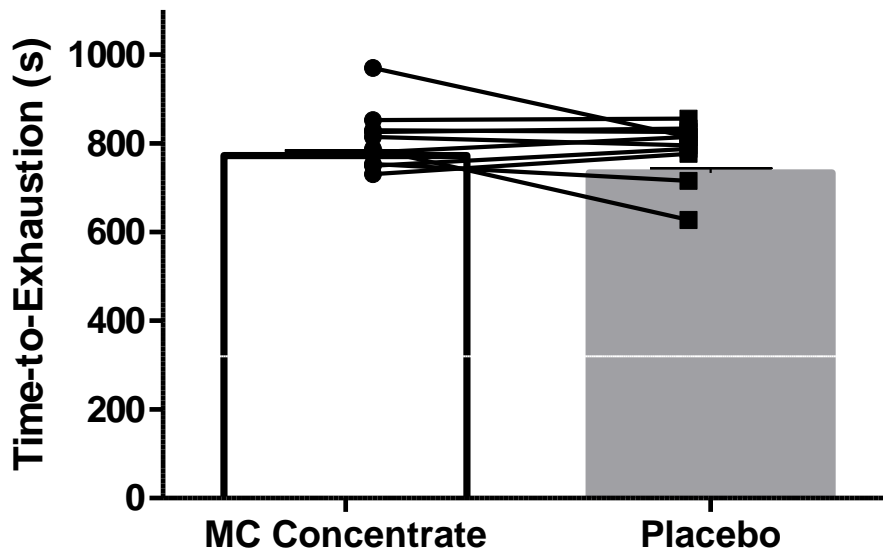
	MC Concentrate	Placebo
<i>Moderate-intensity exercise</i>		
$\dot{V}O_2$, l/min		
Baseline	1.67 ± 0.09	1.68 ± 0.11
End-exercise	2.22 ± 0.09	2.28 ± 0.10
Fundamental amplitude, l/min	0.55 ± 0.09	0.60 ± 0.07
<i>Severe – intensity exercise</i>		
$\dot{V}O_2$, l/min		
Baseline	1.72 ± 0.04	1.74 ± 0.04
360 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

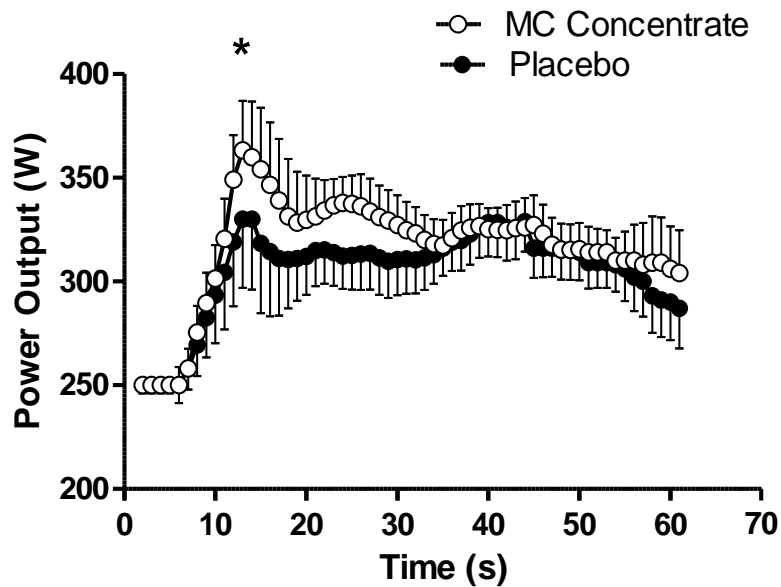
290 The time to exhaustion during the severe-intensity constant-work-rate cycle trials (the exercise
 291 tolerance test) are presented in Fig 1 and while the power profiles for the two experimental
 292 conditions during the 60-s all-out sprint that followed the 6-min bout of severe intensity
 293 exercise (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).



301

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 \pm SEM.

305



306

307 Fig 2 - Group mean power profiles during a 60s 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 \pm SEM.

311

312 **NIRS**

313 The tissue oxygenation index data during moderate- and severe-intensity cycle exercise with
 314 PLA and MC supplementation are reported in Table 2. There were no significant differences
 315 between the experimental conditions during the moderate or severe-intensity exercise ($P >$
 316 0.05).

317

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

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

320 All values are means ± SEM.

321

322 **Vascular measures**

323 There was a significant interaction effect for supplement on SBP ($P < 0.05$), with follow-up
 324 analyses showing that SBP was lower 1.5 h post MC supplementation, with reductions of 5 ±
 325 2 mmHg compared to the placebo trial. No other vascular variables (DBP, mean arterial
 326 pressure (MAP), PWV, augmentation index (AIx) and AIx corrected for HR at 75 bpm) were
 327 altered after consumption of the MC concentrate compared to the placebo treatment. The
 328 absolute values for all variables are presented in Table 3.

329

330

331 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

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

336

337 **Plasma [NO₂⁻] and [NO₃⁻]**

338 Due to sampling error, blood was analysed in 8 participants. The plasma [NO₂⁻] and [NO₃⁻]
 339 for the MC and PLA conditions are reported in Table 4. There were no changes for NO₂⁻ or
 340 NO₃⁻ in the MC supplemented trial when compared to the placebo (P > 0.05).

341

342

343 Table 4 - Plasma [NO₂⁻] and [NO₃⁻] at baseline and 1.5 h following MC concentrate and PLA
344 supplementation.

	MC Concentrate		PLA	
	Pre	Post	Pre	Post
Plasma [NO₂⁻] nM	65 ± 9	68 ± 8	63 ± 7	63 ± 9
Plasma [NO₃⁻] μM	19 ± 2	18 ± 2	20 ± 2	18 ± 2

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

346 **Lactate**

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

351

352

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

Lactate (mmol/L)	Time points				
	1	2	3	4	5
	<i>Exercise performance test</i>				
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†
	<i>Exercise tolerance test</i>				
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†

355
 356 All values are means ± SEM (n=10). † Significant difference between time points from
 357 baseline, P < 0.05.

358

359 Discussion

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

365 *Influence of MC supplementation on performance*

366 In the current study, peak power output and total work done during a 60-s sprint completed
 367 immediately following 6 min of severe-intensity exercise was increased by 9.5 and 10%
 368 respectively, following MC relative to the PLA supplementation. While tart cherry
 369 supplementation has been shown to improve exercise recovery^{22,23}, decrease markers of
 370 inflammation and oxidative stress^{1,3}, studies investigating the effects of tart cherries on
 371 exercise performance are limited and equivocal. Of the two studies investigating the influence
 372 of MC supplementation on exercise performance to date, one reported improved performance
 373 in males completing a half marathon (21.1km) run, as evidenced by a faster overall race pace
 374 compared to the PLA group¹⁹. While Levers et al. designed an experiment to assess the
 375 influence of ingesting 480 mg of powdered tart cherries for 10-days, including supplementation

376 on race day up to 48-hr post-run, we investigated the effects of a single dose (60 ml) of MC
377 concentrate on exercise performance using a cross over study design. Despite the differences
378 in dosing strategies, both studies reported improvements in performance in trained participants.
379 Therefore, our findings suggest that acute as well as chronic supplementation with MC
380 concentrate has the potential to improve endurance performance, specially end-sprint
381 performance. Conversely, an earlier study by Clifford and colleagues ¹⁸ reported no difference
382 in time trial performance in moderately-trained individuals following the ingestion of 200 mg
383 of powdered tart cherries for 3-days. These conflicting findings are likely linked to the
384 differences in dosing procedures (480mg of providing 991mg of phenolic compounds versus
385 200mg of providing 216 mg of polyphenols) and exercise performance tests (20 km cycling
386 time trial versus half marathon). There were no differences observed for time to exhaustion
387 between the MC and the PLA trial in the current study. There remains a debate surrounding
388 the applicability and repeatability of the time to exhaustion test ³¹. However, a recent addition
389 to the literature highlighted that cycling performance is superior for time to exhaustion versus
390 time trial and therefore should not be disregarded as a useful measure of performance in the
391 laboratory ³².

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

409 ^{1,2}, it makes the argument tenable that the improvement in exercise performance in the current
410 study might be as a result of these AOX compounds. It is worth commenting that MC
411 supplementation could have prolonged the duration for which the participants were in the
412 optimal cellular redox state for force production ³⁵ such that when they were required to
413 produce an all-out sprint, they produced a higher peak power and completed more work.
414 Furthermore, this improvement in exercise performance might also be as a result of an increase
415 in blood flow, as previous research has demonstrated the positive effects of MC
416 supplementation on vascular function, ^{10,11} that may help to overcome any potential circulatory
417 limitations following strenuous exercise, attenuating the diminishing O₂ supply to the
418 exercising muscles and maintaining force production during the final 60s ³⁶.

419 *Influence of MC supplementation on plasma [NO₂⁻]*

420 Nitric oxide is a key regulator of vascular integrity. This multifaceted physiological signalling
421 molecule can be synthesized endogenously through NOS with plasma [NO₂⁻] reflecting NOS
422 activity ²⁵. No significant difference in plasma [NO₂⁻] was reported between the MC and PLA
423 trials in the current study. This is somewhat in agreement with the findings from Keane and
424 colleagues ¹¹, where no main effect for plasma nitrate or nitrite was observed following 60 mL
425 MC supplementation using an ELISA kit. Importantly, the lack of a change in plasma [NO₂⁻]
426 in the current study extends our previous findings by using a more sensitive method to detect
427 plasma [NO₂⁻] in the nM range and this better reflects NOS activity than plasma [NO₃⁻] ²⁵.
428 Since trained endurance cyclists were recruited in the current study, and since endurance
429 training increase NOS expression ³⁷; it is likely that eNOS-derived NO is already functioning
430 optimally in this cohort and therefore no changes were observed after MC supplementation. It
431 is also interesting to note, the resting plasma NO₂⁻ levels are quite low in the current study when
432 compared with previous literature ^{30,38}, this could be as a result of the strict dietary restrictions
433 imposed on the participants on the day preceding the trial or a low intake of nitrate-rich foods
434 in general.

435 *Influence of MC supplementation on blood pressure*

436 A primary outcome of enhanced NO synthesis is a reduction in blood pressure owing to NO-
437 induced smooth muscle relaxation ³⁹. The current study reported a significant reduction in SBP
438 1.5 h post MC ingestion relative to placebo, however this augmented modulation occurred in
439 the absence of changes in NO biomarkers. These results are consistent with recent studies
440 demonstrated that supplementation with the NOS substrates, L-Citrulline ³⁰ or L-arginine ³⁸,

441 lowered blood pressure in the absence of a change in plasma $[\text{NO}_2^-]$. Mechanistically, it would
442 appear that the lowering of BP with acute MC supplementation in the current study is largely
443 NO-independent and is more likely to be a function of the increase in circulating phenolic
444 metabolites post MC ingestion ¹¹.

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

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

458 In conclusion, this study has shown that acute supplementation with MC juice can lower blood
459 pressure and improve exercise performance, specifically end-sprint performance, in trained
460 endurance cyclists. There were no changes in plasma $[\text{NO}_2^-]$, pulmonary $\dot{V}\text{O}_2$, or muscle
461 oxygenation after ingesting tart cherry juice so the improvements in blood pressure and
462 exercise performance in this study might be mediated through the potent antioxidant properties
463 of MC juice. The results of this study suggest that supplementation with MC concentrate might
464 represent, a practical, non-pharmacological, dietary intervention to reduce blood pressure and
465 enhance end-sprint performance in trained individuals.

466 **Perspectives**

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

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

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