1 **Tart Cherry Supplement Enhances Skeletal Muscle Glutathione Peroxidase Expression**
2 **and Functional Recovery After Muscle Damage**
3
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19

Abstract

Introduction

Montmorency cherry concentrate (MCC) supplementation enhances functional recovery from exercise, potentially due to antioxidant and anti-inflammatory effects. However, to date, supporting empirical evidence for these mechanistic hypotheses is reliant on indirect blood biomarkers. This study is the first to investigate functional recovery from exercise alongside molecular changes within the exercised muscle following MCC supplementation.

Methods

Ten participants completed two maximal unilateral eccentric knee extension trials following MCC or placebo supplementation for 7 days prior to and 48 hours following exercise. Knee extension maximum voluntary isometric contractions (MVC), maximal isokinetic contractions, single leg jumps, and soreness measures were assessed before, immediately, 24 and 48 h after exercise. Venous blood and *vastus lateralis* muscle samples were collected at each time point. Plasma concentrations of IL-6, TNF-α, C-reactive protein, creatine kinase, and phenolic acids were quantified. Intramuscular mRNA expression of SOD 1 and 3, GPX1, 3, 4 and 7, Catalase, and Nrf2 and relative intramuscular protein expression of SOD1, Catalase and GPX3 were quantified.

Results

MCC supplementation enhanced recovery of normalized MVC 1s average compared to placebo (Post- Exercise PLA: 59.5±18.0% vs MCC: 76.5±13.9%; 24 h PLA: 69.8±15.9% vs MCC: 80.5±15.3%; supplementation effect p=0.024). MCC supplementation increased plasma hydroxybenzoic, hippuric and vanillic acid concentrations (supplementation effect p =

0.028, p = 0.002, p= 0.003); SOD3, GPX3, GPX4, GPX7 (supplement effect $p < 0.05$) and GPX1 (interaction effect p = 0.017) gene expression; and GPX3 protein expression (supplementation effect p = 0.004) versus placebo. There were no significant differences between conditions for other outcome measures.

Conclusion

MCC supplementation conserved isometric muscle strength and upregulated antioxidant gene and protein expression in parallel with increased phenolic acid concentrations.

Keywords: Phenolic Acids, Resistance Exercise, Muscle Damage, Antioxidant, Oxidative Stress

Abbreviations

Akt (Protein Kinase B)

ANOVA (Analysis of Variance)

BCA (Bicinchoninic Acid)

βME (Beta-mercaptoethanol)

BSA (Bovine Serum Albumin)

CAT (Catalase)

cDNA (complementary Deoxyribonucleic Acid)

CK (Creatine Kinase)

CRP (C-Reactive Protein)

ddH_2O (double-distilled water)

ECL (Enhanced Chemiluminescence)

EC^{Max} (Maximal Eccentric Knee Flexion Contractions)

70 EDTA (Ethylenediaminetetraacetic Acid)

71 ELISA (Enzyme-linked Immunosorbent Assay)

72 GPX 1,3,4,7 (Glutathione Peroxidase 1,3,4,7)

73 High performance liquid chromatography (HPLC)

74 $IKCon^{Max}$ (IK^{Max} Concentric Phase)

75 $IKEcc^{Mac}$ (IK^{Max} Eccentric Phase)

76 IK^{Max} (Maximal Isokinetic Knee Extension and Flexion)

77 IL-6 (Interleukin-6)

78 Keap1 (Kelch-like ECH-associated protein 1)

79 LSD (Lithium Dodecyl Sulphate)

80 MCC (Montmorency Cherry Concentrate)

81 mRNA (messenger Ribonucleic Acid)

82 mTOR (Mammalian Target of Rapamycin)

83 MVC (Maximal Voluntary Isometric Contraction)

84 NADPH (Nicotinamide Adenine Dinucleotide Phosphate)

85 Nrf2 (Nuclear factor erythroid 2-related factor 2)

86 PARQ (Physical Activity Readiness Questionnaire)

87 PLA (Placebo)

88 PPT (Pain Pressure Threshold)

89 $PPT^{VL/VM/RF/SUM}$ (Pain Pressure Threshold at Vastus Lateralis/Vastus Medialis/Rectus

90 Femoris/sum of VL, VM and RF)

91 RIPA (Radioimmunoprecipitation Assay)

92 RNA (Ribonucleic Acid)

93 ROM (Range of Motion)

94 ROS (Reactive Oxygen Species)

95 rRNA (ribosomal Ribonucleic Acid)

96 RTqPCR (Real-Time Polymerase Chain Reaction)

97 SLJ (Single Leg Jump)

98 SOD 1, 2, 3 (Superoxide Dismutase 1, 2, 3)

99 SR (Sarcoplasmic Reticulum)

100 TBS (Tris-buffered Saline)

101 TBST (TBS with tween)

102 TNFα (Tumour Necrosis Factor Alpha)

103 VAS (Visual Analogue Scale)

104 **Introduction**

105

106 Intense exercise may induce muscle damage, resulting in muscle soreness and associated
107 reductions in force generating capacity of the muscle. This damage occurs through a complex
108 combination of mechanisms including structural damage to the contractile apparatus, as well
109 as disruption to biochemical pathways such as those governing skeletal muscle calcium
110 handling. This is due, in part, to high intra-muscular forces and increased reactive oxygen
111 species (ROS) exposure, generated during exercise (1, 2).

112

113 ROS are generated during exercise (1, 2) and are understood to play an important role in
114 maintaining homeostasis; when levels exceed the capabilities of the endogenous antioxidant
115 defence mechanisms, cellular redox balance is altered resulting in oxidative stress (3). This in
116 turn causes further disruption and damage to cellular processes and structures (1-4). For
117 example, structures within the sarcoplasmic reticulum (SR) are sensitive to ROS, such that
118 increased exposure to ROS impairs muscle calcium handling and sensitivity (5). This leads to
119 decrements in muscle contractile force development and consequently exercise performance
120 (2, 4). Furthermore, disruptions in skeletal muscle calcium handling, are also likely to impair
121 recovery; for example via elevating muscle protein breakdown and reducing phosphorylation
122 of protein kinase B (Akt) and mammalian target of rapamycin (mTOR) (6), thereby reducing
123 protein synthesis required for repair.

124

125 Due to this involvement of oxidative damage, there has been an abundance of research
126 investigating the use of exogenous antioxidant supplements as a means of reducing exercise
127 induced muscle damage and the associated recovery time (7, 8). Numerous studies have
128 shown that polyphenol supplementation reduces blood markers of oxidative damage and

129 inflammation (9-13). Montmorency cherries contain high concentrations of polyphenols (14),
130 and there is evidence that their consumption in supplement form may attenuate oxidative
131 stress, inflammation and muscle soreness; aiding muscular recovery from multiple exercise
132 modalities (9-11, 13, 15, 16). However, to-date, research in this area has relied on proxy
133 markers of intra-muscular oxidative stress and inflammation within blood plasma or serum,
134 rather than analysis of the exercised muscle tissue itself. This approach is unlikely to
135 comprehensively elucidate the effects of intensive exercise and supplementation strategies to
136 support recovery, since these proxy measures have been shown to respond differently in
137 recovery from intensive exercise to direct muscle measures (17).
138
139 The mechanisms by which supplementation may enhance recovery are unclear, and the
140 limited evidence available is equivocal. Initial theories proposed radical scavenging as the
141 primary mechanism, due to the ability of phenolic compounds to donate electrons via
142 hydrogen atom transfer from a hydroxyl unit. However, the low concentrations of
143 polyphenols and phenolic metabolites present in the plasma suggest they are unlikely to act
144 as direct antioxidants *in vivo* (18). We and others have hypothesised that it is more likely that
145 antioxidant effects *in vivo* arise from nuclear translocation and activation of Nuclear Factor
146 Erythroid 2–related Factor-2 (Nrf2) signalling following exposure to phenolic metabolites (7,
147 19, 20).
148
149 This study is the first to investigate the potential biological mechanisms within human
150 skeletal muscle that underpin improvements in exercise recovery in response to
151 Montmorency cherry concentrate (MCC) supplementation. We quantify antioxidant mRNA
152 and protein expression within exercised muscle tissue. We also quantify the phenolic
153 metabolites of MCC in plasma throughout the MCC loading and exercise recovery phases. It

154 was hypothesised that supplementation would amplify gene and protein expression of
155 endogenous antioxidant enzymes thus enhancing functional recovery.
156
157 **Methods**
158
159 This study employed a double-blind cross-over design in which participants completed two
160 trials separated by a two-week washout period in line with previous literature (10, 15, 21, 22).
161 The study received ethical approval from the Sport and Health Sciences ethics committee at
162 the University of Exeter and Human Research Ethics committee at the University of
163 Queensland and all testing conformed to the guidance set out by the Declaration of Helsinki
164 (see additional information for details).
165
166 **Participants**
167
168 Ten recreationally active male participants (age= 23.4±5.4 years, weight=78.0±21.9 kg,
169 height= 178.4±6.9 m), asymptomatic of illness and injury, completed the study. Twelve
170 participants were recruited and consented, but two withdrew prior to completion of the study.
171 One participant was unable to participate following consent and familiarisation, due to
172 commitment to another study which required dietary manipulation, and one participant
173 completed one arm of the study but was un-responsive to all subsequent contact attempts.
174 Participants completed a physical activity readiness questionnaire (PARQ) and medical and
175 exclusion screening questionnaire, before providing written informed consent. PARQ was
176 also used to exclude sedentary individuals. Exclusion criteria comprised individuals aged
177 below 18 or above 40 years, females, individuals allergic to fruit, and highly trained
178 individuals. Females were excluded to avoid any confounding influence of low-grade

179 inflammation caused by menstrual cycle symptoms (23), and highly trained individuals were
180 excluded due to their familiarity with high intensity exercise, which may have dampened the
181 effects of the exercise damage protocol (24). Individuals who are highly trained will have
182 chronic training adaptations, such as increased antioxidant and buffering capacity, as well as
183 increased monocarboxylate transporters, which can improve the speed of post-exercise
184 recovery (25). Furthermore, trained individuals' familiarity with eccentric exercise may be
185 protective against muscle damage from subsequent eccentric exercise during the damage
186 protocol (26). Trained individuals were excluded by verbal questioning prior to giving
187 informed consent, and their responses were subsequently confirmed by visual assessment of
188 their activity diary. Trained individuals were defined as completing more than 3 h.wk-1 of
189 deliberate planned exercise outside of normal physical activity as defined by Caspersen *et al.*
190 (27).
191
192 Sample size was calculated as 10 participants to provide 80% power to detect a 5% difference
193 between trials, based on the expected difference between MCC and placebo (PLA) (effect
194 size of 1). Calculations were based on the maximum voluntary isometric contraction (MVC)
195 force recovery data from Bowtell *et al*. (10) and the anticipation of a curvilinear relationship
196 between dosage and functional effects on performance markers. 12 participants were initially
197 recruited to account for dropouts.
198
199 **Supplementation Period**
200
201 Trials were counterbalanced for trial order and leg dominance, with participants and
202 investigators blinded to treatment to avoid potential bias. A researcher, who had no further
203 involvement with data collection, prepared the supplement packs for the participants. The

204 participants were randomised to supplement code 'A' or 'B' using a sealed envelope system.
205 The MCC and PLA supplements were then provided to investigators by a member of the
206 research team not involved in data collection, in opaque bags coded 'A' or 'B.'
207
208 During each trial participants ingested two 30 mL daily doses (morning and evening) of
209 either MCC or PLA, for ten days. MCC supplement was a commercially available product
210 (CherryActive®, ActivEdge, U.K) produced from US Montmorency cherries. PLA was a
211 commercially available fruit concentrate (Morello Cherry Cordial, Blossom Cottage,
212 Gloucester, U.K) with additional carbohydrate added to ensure it was isoenergetic. Analysis
213 of the phenolic content of the MCC by high performance liquid chromatography (HPLC) (28)
214 was conducted by Atlas Bioscience, Inc. (Tucson, AZ). Total content was 20.167 $mg \cdot mL^{-1}$
215 polyphenolics, 7.211 $mg \cdot mL^{-1}$ total anthocyanins, with pelargonidin (3.319 $mg \cdot mL^{-1}$) and
216 delphinidin (1.299 $mg \cdot mL^{-1}$) the most prevalent anthocyanins (see Table A, SDC, for details
217 on polyphenol composition of MCC supplement). This supplementation protocol has
218 previously been shown to enhance recovery of MVC knee extensors following exercise
219 induced muscle damage (10). Participants were also asked to maintain any normal exercise
220 habits throughout the trial, but to refrain from high volume and high intensity exercise, such
221 as resistance, interval or unaccustomed endurance type exercise, for 48 hours prior to
222 experimental visits. Participants were also asked to maintain their normal diet, but to avoid
223 any increase in consumption of foods with high polyphenol concentrations during the
224 supplementation and wash out periods, in addition to alcohol and caffeine 48 hours before the
225 test. To this end, participants were provided with diet and exercise diaries, to record all food
226 and beverages consumed in the final 6 days of the supplementation period, and exercise
227 completed over the final five days of each supplementation period. Avoidance of an increase
228 in consumption of polyphenol rich foods, rather than avoidance *in toto* was advised in order

229 to maintain ecological validity. Participants were also asked to refrain from eating or drinking
230 anything apart from water for 10 h prior to the laboratory visits during both supplementation
231 periods, and all lab tests were conducted at the same time of day for each participant. On days
232 where experimental testing occurred, morning doses of MCC were consumed by participant
233 prior to arrival at the laboratory.
234
235 **Experimental Design**
236
237 **Familiarisation**
238
239 Participants' baseline measures of height and weight were assessed, before familiarisation
240 with all experimental procedures, and measurement of MVC. Performance of the warm-up
241 during familiarisation was force limited to 100 N for all participants. Familiarisation for all
242 tests occurred for both dominant and non-dominant legs on the same day. A maximum of one
243 set of the damage protocol was performed sub-maximally. Chair and dynamometer arm
244 settings for the Biodex were determined during the familiarisation visit and recorded for use
245 in all subsequent tests. Leg dominance was determined by defining the non-dominant leg as
246 the stabilising leg during single leg movements such as kicking.
247
248 **Damage Protocol and Functional Testing**
249
250 Overnight fasted participants returned to the laboratory on day 8 of supplementation at which
251 point resting venous blood samples were collected from an antecubital vein. Serum samples
252 were collected in tubes containing clot activator and gel for serum separation and kept at
253 room temperature for 30 minutes, and plasma samples were collected in tubes containing

254 lithium heparin. Tubes were centrifuged at 4500 rpm for 15 minutes at 4°C to fractionate
255 samples and remove the cellular components. Serum and plasma were distributed into
256 microcentrifuge tubes before storage at -80°C until analysis. *Vastus lateralis* muscle biopsy
257 samples were taken using the suction-modified percutaneous Bergstrom needle technique
258 (29). The leg from which the biopsy was taken was sterilised using iodine and anaesthetised
259 locally with 2% lidocaine. An incision of approximately 0.8 cm was then made, before a
260 biopsy needle was used to collect a sample of muscle (~150 mg). The incision was then
261 closed with butterfly stitches and covered with a waterproof dressing. Eight biopsies were
262 taken from each participant, with four per experimental trial, prior to and after exercise, at 24
263 and 48 hours. All biopsies were taken from the same leg during the course of each trial, each
264 time from a new incision.
265
266 Muscle soreness was assessed with participants seated and knee extensors in a stretched
267 position, with a knee angle of 90° via the use of a (200 mm) visual analogue scale (VAS) and
268 pain pressure threshold (PPT) using a handheld algometer (FDX 50, Wagner, Greenwich, CT
269 06836-1217 USA) (10, 16). Algometer measures were taken by application of increasing
270 pressure, with a handheld algometer, to the participant determined point of being
271 'uncomfortable but not painful,' at the vastus lateralis, vastus medialis and rectus femoris.
272 One measure was taken at each site by the same investigator before, post, and 24 and 48
273 hours after completion of the damage protocol as in Bowtell *et al.* (10). For VAS analysis
274 participants were instructed to mark their level of soreness on a line from 0 to 10, with 0
275 being no pain, and 10 being extreme pain. VAS of this length have previously been shown to
276 have good reliability for measuring acute pain and detecting changes in pain intensity (30).
277

278 The exercise protocol (Figure 1) consisted of a warm-up, muscle function measures (single-
279 leg maximal isokinetic knee extension and flexion repetitions (IK^{Max}); MVC, single leg
280 jumps (SLJs)) and a muscle damage protocol, using a Biodex Isokinetic Dynamometer
281 (Biodex System 3 Medical Systems 830-200, Shirley, N.Y. 11967 USA). Functional
282 measures and muscle damaging exercise via eccentric contractions of knee extension exercise
283 were selected in line with previous research to allow for direct comparisons (10, 31).
284
285 Before beginning experimental measures (IK^{Max}, MVC and SLJ), participants completed the
286 warm-up protocol using the leg from which the pre-exercise biopsy had been taken. The
287 warm-up consisted of 5 sets of 5, single-leg sub-maximal isokinetic knee extension and
288 flexion repetitions with a force limit set at 50% of familiarisation MVC for that leg, separated
289 by 1 minute of rest. Following the warm-up protocol, participants completed, 3 sets of 3
290 IK^{Max} repetitions and 3 MVCs, separated by 1 minute of rest, and 3 SLJs performed
291 consecutively with a rest period of at least 10 seconds. Jumps were performed on a mat
292 (Jump Mat Pro, SL Electronics Ltd., Cookstown, UK) with hands on hips and a single leg
293 take-off to isolate performance as much as possible to the limb of interest. A two-footed
294 landing was used to account for discrepancies in participants ability to balance upon landing,
295 especially with fatigue following the exercise protocol. Jump height was recorded in mm.
296
297 Warm up and IK^{Max} repetitions were performed over a range of motion (ROM) of 80° from
298 full flexion at the knee, at $60° \cdot s^{-1}$ for both the concentric and eccentric phases. MVC
299 repetitions were performed at a knee angle of 90°, with the Biodex arm stationary, as this has
300 previously been shown to be the angle at which the maximum amount of force can be
301 produced, due to optimal overlap of sarcomeres (32).
302

303 The damaging exercise protocol was performed 5 minutes after completion of SLJs, and
304 consisted of 10 sets of 30 maximal eccentric knee flexion contractions (EC^{Max}), with each set
305 separated by a period of 1-minute. Repetitions were performed over the same ROM of 80° as
306 that used for the warm-up and IK^{Max} repetitions; with a passive (no contraction) concentric
307 phase at $180°·s^{-1}$, and a maximal eccentric phase, at $60°·s^{-1}$. There were no significant
308 differences in joint angles between legs ($p = 0.528$). Performance tests were then repeated
309 following the damage protocol. Throughout the experimental measures and damage protocol,
310 participants were given verbal encouragement. A further muscle biopsy and blood sample
311 were taken immediately following completion of the post-damage protocol performance tests,
312 in addition to further measures of muscle soreness.
313
314 Twenty-four and forty-eight hours later, participants returned to the laboratory following an
315 overnight fast. During both visits, resting venous blood samples, a further assessment of
316 muscle soreness, and a further muscle biopsy were taken, before repetition of the warm-up,
317 functional performance measures (Figure 1). All biopsies were taken from the same leg
318 during the course of a trial. Following a two-week supplement wash-out period, this protocol
319 was repeated with the functional measures and damage protocol performed using the
320 contralateral leg, in order to minimise any repeated bout effects. All visits for each participant
321 were performed at the same time of day.
322
323 **Force Recordings**
324
325 Force produced during knee extension exercise was measured using a Biodex isokinetic
326 dynamometer. Torque was displayed in Newton metres (Nm). Force data were recorded and
327 analysed using a custom written script in Spike2 ver.6 software (CED, Cambridge, UK).

328

329 Work done during the damaging exercise protocol was determined via calculation of the area

330 under the force time curve:

331

332 $Work = \text{force} \times \text{time trace}$

333

334 Force data from MVCs were analysed to calculate both peak force output and the highest

335 average value over a 1 second period, occurring within the plateau of each contraction. The

336 reported MVC value for each respective time point was represented by the highest value

337 achieved across the 3 MVC contractions for each measurement time point to ensure the

338 maximal possible peak values was recorded.

339

340 Force data for maximal isokinetic contractions were assessed by measurement of peak force

341 during each set for the three individual concentric and eccentric contractions respectively

342 completed during each set at each measurement time point. Three sets of contractions were

343 completed at each time point, from which the highest values of peak concentric and eccentric

344 force were taken.

345

346 **Sample Analyses**

347

348 **Blood Sample Analysis**

349

350 Serum samples were analysed for activity of Interleukin-6 (IL-6); C-reactive protein (CRP),

351 and Tumour Necrosis Factor Alpha (TNF- α) via ELISA (IL-6: HS600C; TNF- α: HSTA00E;

352 CRP: DCRP00, R&D Systems Quantikine High Sensitivity ELISA, R&D Systems,

353 Minneapolis, United States), according to the manufacturer's instructions to assess muscle
354 damage and systemic inflammation. Creatine Kinase (CK) analysis was performed by the
355 Royal Devon and Exeter NHS Trust on the 702 module of the Cobas 8000 automated
356 platform according to the manufacturers recommended protocol (Roche Diagnostics, Basel,
357 Switzerland).
358
359 **Measurement of Plasma Phenolic Metabolites Profile by High-Resolution Accurate-**
360 **Mass (HRAM) Mass Spectrometry**
361
362 Phenolic metabolite analysis were performed at the Bioanalytical Facility, University of East
363 Anglia. Plasma concentrations of protocatechuic acid, 4-hydroxybenzoic acid, hippuric acid,
364 vanillic acid, ferulic acid and isoferulic acid were quantified using an Orbitrap Velos Linear
365 Trap Quadropole (LTQ) high-resolution accurate-mass (HRAM) mass spectrometry system
366 coupled with an Accela autosampler and ultra high-pressure liquid chromatography pump
367 (Thermo Scientific, Cheshire, UK). The Orbitrap system was operated in Fourier transform
368 MS mode at the resolution of 30,000 in negative electrospray ionisation (ESI) mode.
369
370 To prepare the samples for analysis, 200 µL of plasma/calibration stands/quality controls and
371 20 µL of internal standard containing ferulic acid-[2H3] (100 nmol.L-1) and hippuric acid
372 [13C6] (200 µmol.L-1) (Toronto Research Chemicals, Ontario, Canada). in 0.1% formic acid
373 (Merck, Germany), were pipetted into a microcentifuge tube and mixed. To this, 1 mL of
374 methanol was added slowly with gentle mixing, the mixture was then incubated at room
375 temperature for 15 minutes, followed by centrifugation at 14000 rpm for 7 minutes. The
376 supernatant was transferred to borosilicate glass tube and placed in an evaporator to dry
377 under a constant stream of nitrogen at a temperature of 60°C. To the dried supernatant 200

378 μL of methanol (Merck, Germany) with 0.1% formic acid was added into each tube and
379 vortex mixed for 30 seconds, followed by 2.5 mL of ethylacetate (Merck, Germany) and
380 vigorously mixed for 10 minutes. After centrifugation at 4000 rpm for 10 minutes, 2 mL of
381 the ethylacetate in the upper layer was transferred to a fresh set of borosilicate glass tubes and
382 again evaporated to dryness as described above. The dried residue was resuspended in 250
383 μL of LCMS grade deionised water with 1% acetic acid (Merck, Germany), then vortex
384 mixed followed by centrifugation at 4000 rpm for 10 mins. The final mixture was transferred
385 into polypropylene autosampler vials, 50μL was injected into the liquid chromatography
386 high-resolution mass spectrometry system for analysis.
387
388 Chromatographic separation was achieved using a ModusCore C18 reverse phase column
389 (2.1 m x 50 mm, 2.7 μm) (Chromatography direct, Runcorn, UK) maintained at a
390 temperature of 40°C. Mobile phases A consisted of 1% acetic acid in LCMS grade deionised
391 water with LCMS grade methanol as mobile phase B. The binary gradient program was: 0
392 min 1% B, 0–1 min 1% B with a linear increase to 45% B at 10 min, 10-10.5 min 95% B and
393 held to 12 min, returned to 1% B at 12.5 min to re-equilibrate with a cycle time of 15 mins.
394 Mobile phase flow rate was 0.5 mL per min throughout the run.
395
396 The mass scan range used to quantify each phenolic metabolite was determined by direct
397 infusion of pure standards into the ion source via a T-connector. European Pharmacopoeia
398 (EP) reference standards used to prepare the calibration standards were obtained via Merck
399 (Germany). The quantitation mass range (Da) for protocatechuic acid (152.99800-
400 153.00300), 4-hydroxybenzoic acid (137.005-137.010), hippuric acid (178.026-178.032),
401 vanillic acid (167.012-167.017), ferulic acid and isoferulic acid (193.023-193.030). Xcalibur
402 software version 2.1 (Thermo Scientific, Cheshire, UK) were used for system control, data

403 acquisition, baseline integration and peak quantification (see Table B, SDC, for summary of
404 assay performance).
405
406 **Muscle Sample Analysis**
407
408 Muscle samples were analysed for protein concentrations of SOD1, Catalase, GPX3, GPX4
409 and GPX7, as well as gene expression of SOD1, SOD2, SOD3, GPX1, GPX3, GPX4, GPX7,
410 Catalase, and Nrf2.
411
412 **Gene Expression (Real-Time Quantitative Polymerase Chain Reaction (RTqPCR))**
413
414 Superoxide dismutase (SOD) 1, SOD2, SOD3, Glutathione Peroxidase (GPX) 1, GPX3,
415 GPX4, GPX7, Catalase (CAT), and Nrf2 mRNA expression were quantified using TaqMan®
416 Array 96-Well Fast Plates. RNA was extracted from muscle samples by immersion in 500 µL
417 of TRIzol reagent (Sigma-Aldrich Company Ltd., Dorset, United Kingdom), and bead
418 homogenisation (Speedmill Plus, Analytik Jena AG, Jena, Germany) for 30 seconds and 1
419 minute sequentially. RNA was extracted according to the TRIsol manufacturer's instructions
420 and was resuspended in 30 µL nuclease free water (Severn Biotech, Limited, Kidderminster,
421 United Kingdom). Samples were heated for 5 minutes at 60°C to ensure complete
422 solubilisation.
423
424 Subsequently, RNA concentration and purity of samples was analysed by spectrophotometry
425 (NanoDrop Lite Spectrophotometer, ThermoFisher Scientific, Waltham, Massachusetts,
426 United States), before cDNA transcription of RNA was performed with Primerdesign

427 Precision nanoScript 2 Reverse Transcription kit, according to manufacturer's instructions
428 (Primerdesign, Southampton, United Kingdom).
429
430 Following this, 5 μL of TaqMan fast advanced master mix (Applied Biosystems, Waltham,
431 Massachusetts, United States) and 5 μL of each sample containing 2.5ng cDNA were added
432 to custom TaqMan Gene Expression Array 96 well fast plates (Applied Biosystems,
433 Waltham, Massachusetts, United States). Plates were then sealed and vortexed briefly to
434 ensure contents were mixed, before centrifuging for 1 minute at 1200 g at 4°C (Sorvall ST 16
435 Centrifuge Series, ThermoFisher Scientific, Waltham, Massachusetts, United States). Plates
436 were then loaded into the RTqPCR instrument for analysis (QuantStudio 6 Flex Real-Time
437 PCR System, ThermoFisher Scientific, Waltham, Massachusetts, United States). Samples
438 underwent 1 cycle for enzyme activation at 95°C for 20 seconds; and then underwent 40
439 cycles of sequential denaturing at 95°C for 1 minute, and annealing/extending at 60°C for 20
440 seconds. The internal control used was 18s rRNA (data available in SDC Table C).
441
442 RTqPCR fold change was calculated using the Pfaffl formula (33) for quantification relative
443 to the pre-exercise placebo condition. These values were $\log_{10}(x)$ transformed prior to
444 analysis in order to linearise data. Primer efficiency was assumed to be 2.
445
446 **Protein Content Analysis**
447
448 *Protein Extraction*
449
450 25 mg muscle was placed in microcentrifuge tubes with 400 μL of radioimmunoprecipitation
451 assay (RIPA) buffer (1 Pierce A32961 Protease and Phosphatase Inhibitor EDTA-free mini

452 tablet, ThermoFisher Scientific, Waltham, Massachusetts, United States, dissolved in 10 mL
453 Pierce 89900 RIPA buffer, ThermoFisher Scientific, Waltham, Massachusetts, United
454 States), before bead homogenisation (Speedmill Plus, Analytik Jena AG, Jena, Germany) for
455 30 seconds and 1 minute sequentially. Homogenised muscle and RIPA buffer samples were
456 then aspirated and placed into clean microcentrifuge tubes and vortexed thoroughly
457 (FB15012 TopMix Vortex Mixer, ThermoFisher Scientific, Waltham, Massachusetts, United
458 States), before incubation on ice for 30 minutes, with occasional vortexing. Samples were
459 then centrifuged for 10 minutes at 8000 g at 4°C (Sorvall ST 16 Centrifuge Series,
460 ThermoFisher Scientific, Waltham, Massachusetts, United States). The supernatant was
461 retained, and the pellet was discarded.
462
463 Protein concentrations were determined by Bicinchoninic acid (BCA) assay (Pierce 23225
464 BCA Protein Assay Kit, ThermoFisher Scientific, Waltham, Massachusetts, United States)
465 according to the manufacturer's instructions. Protein lysate dilutions were then calculated
466 according to values obtained from BCA assay protein content analysis. Samples were diluted
467 in ddH_2O and a 4x 1:0.11, lithium dodecyl sulphate (LSD): β-mercaptoethanol (βME)
468 solution (PCG3009 TruPAGE LDS Sample Buffer, Sigma-Aldrich, St. Louis, Missouri,
469 United States; 2-Mercaptoethanol, ThermoFisher Scientific, Waltham, Massachusetts, United
470 States).
471
472 *Western Blotting*
473
474 Gels were loaded with pre-stained protein molecular weight ladder (Pierce 26612 Prestained
475 Protein MW Marker, ThermoFisher Scientific, Waltham, Massachusetts, United States), a
476 pre-prepared protein standard of pooled positive control sample, produced by combining

477 samples of a dropout participant, and participant lysate samples for analysis. All gel
478 electrophoresis was run at 120 V constant (Mini-PROTEAN Tetra Cell System Tank and
479 PowerPac Basic Power Supply, Bio-Rad, California, United States) until the dye reached the
480 bottom of gels. Membranes were then incubated in a blocking solution of either 5% milk
481 powder (Marvel Dried Skimmed Milk, Premier Foods plc, Hertfordshire, United Kingdom)
482 or 5% BSA (BP9702-100 BSA, Fisher BioReagents, Waltham, Massachusetts, United
483 States), for 1 hour at room temperature before overnight incubation of at least 12 hours in a
484 primary antibody dilution at 4°C (see Table D, SDC, for details on individual protocols by
485 protein target).
486
487 Membranes were incubated in the appropriate secondary antibody dilution at room
488 temperature for 1 hour. The membrane was washed with tris-buffered saline (S5886 Sodium
489 Chloride, Sigma-Aldrich Company Ltd., Dorset, United Kingdom and Tris-Base BP152-1,
490 Fisher BioReagents, Waltham, Massachusetts, United States) (TBS) and Tween 20 (Tween
491 BP337-100, Fisher BioReagents, Waltham, Massachusetts, United States) (TBS-T) solution
492 at least 3 times after every cycle of antibody incubation. Protein detection was conducted on
493 the membrane using enhanced chemiluminescence (ECL) detection reagent (RPN2232
494 Amersham ECL Prime Western Blotting Detection Reagent, GE Healthcare Bio-Sciences,
495 Pittsburgh, United States), and were then imaged for chemiluminescence (ChemiDoc XRS+
496 System, Bio-Rad, California, United States).
497
498 Following imaging, membranes were stained for analysis of protein load using Coomassie
499 blue staining solution (0.25 g C/8540/46 Coomassie Blue R250 powder (Fisher BioReagents,
500 Waltham, Massachusetts, United States) mixed in a solution of 10 mL Glacial acetic acid
501 (A/0360/PB15 Glacial acetic acid, Fisher BioReagents, Waltham, Massachusetts, United

502 States) and 90 mL Methanol:H_2O (1:1 v/v)) (M/4056/15 Methanol, Fisher BioReagents,
503 Waltham, Massachusetts, United States). Membranes were de-stained using TBS-T before
504 colourmetric imaging using automatically determined exposure (ChemiDoc XRS+ System,
505 Bio-Rad, California, United States).

506

507 *Densitometry Analysis*

508

509 Blots were analysed for optical density using ImageJ (Rasband, W.S., ImageJ, U. S. National
510 Institutes of Health, Bethesda, Maryland, USA). Band intensity was normalised to total
511 protein load (Coomassie blue, intensity of entire lane) and normalised across gels using the
512 positive control sample.

513

514 **Statistical Analysis**

515

516 All data (with the exception of total work during the muscle damage protocol for which a
517 paired t-test was used) were analysed by 2-way repeated measures analysis of variance
518 (ANOVA). Where data was missing for one time point in a participant trial arm, z-scores
519 were calculated for the missing data point. In cases where there were multiple data points
520 missing from a participant trial arm, that participant was excluded for the corresponding
521 analysis. MVC data are presented for each time point normalised to pre-exercise MVC to
522 control for differences in pre-exercise MVC between legs and between participants. For
523 transparency, absolute MVC data are also presented along with results of those statistical
524 analyses. Fold change from RTqPCR analysis was linearised by $\log_{10}(x)$ transformation prior
525 to analysis. In cases where a significant interaction effect was detected, post-hoc pairwise
526 tests were conducted with Bonferroni corrections. Throughout analyses, values that did not

527 meet the assumption of sphericity as measured by Mauchly's test, were Greenhouse-Geisser
528 corrected. Where data were not normally distributed and could not be normalised by standard
529 approaches to data transformation, a non-parametric Friedman's test was conducted for
530 comparison with the results of the 2-way repeated measures ANOVA. All statistical analyses
531 were performed using IBM SPSS Statistics (Version 26). For ease of reading, main effects
532 (supplement, time and interaction) and post-hoc differences are only reported in text, tables
533 and figures where statistical significance was achieved.
534
535 **Results**
536
537 Knee extension MVC 1s average decreased to 59.5±18.0% and 76.5±13.9% of pre-exercise
538 for PLA and MCC conditions respectively, following completion of the intensive exercise
539 protocol (Figure 2A). There was no significant difference between conditions in work
540 performed throughout the damage protocol (44722.4 ± 14535.7 J, PLA vs 46812.0 ± 12341.6
541 J, MCC). A visual inspection was conducted on food and activity diaries to ensure
542 participants replicated dietary intake and activity. There was no effect of trial order on the
543 MVC measures (MVC 1s Average: $p = 0.467$, MVC peak: $p = 0.394$).
544
545 MCC supplementation significantly enhanced force recovery of normalised MVC 1s average
546 (supplementation effect, $p = 0.024$, interaction effect, $p = 0.043$) (Figure 2A). Post-hoc
547 testing revealed a significantly higher force recovery for MCC immediately post-exercise (p
548 $= 0.033$), but no significant differences at any other time point.
549
550 Recovery of normalised peak MVC force was enhanced in the MCC versus PLA condition
551 (supplementation effect, $p = 0.032$, interaction effect, $p = 0.049$). Post-hoc testing revealed no

552 significant differences in normalised peak MVC between conditions pre-exercise,

553 immediately, 24- and 48-hours post-exercise, though post-exercise was close to significance

554 ($p = 0.054$) (Figure 2B). Non-normalised peak and 1s average MVC force were not

555 significantly higher in MCC than PLA condition, however there were significant interaction

556 effects for both 1s Average and Peak MVC force (Table 1).

557

558 There was no significant difference in recovery of normalised IK^{Max} (combined eccentric and

559 concentric phases), or during separated concentric ($IKCon^{Max}$) (Figure 2C), or eccentric

560 contraction phases ($IKEcc^{Max}$) (Figure 2D).

561

562 There was no significant difference between supplement conditions in jump height (Figure

563 2E) or soreness measures (VAS and PPT^{VL}, PPT^{VM}, PPT^{RF}, PPT^{SUM}) between conditions

564 (Table 1). Soreness, as measured by VAS and PPT^{RF}, was significantly elevated after the

565 damaging exercise (time effect, $p < 0.05$).

566

567 Plasma hydroxybenzoic acid, hippuric acid and vanillic acid concentrations were

568 significantly higher following MCC supplementation compared with placebo

569 supplementation ($p < 0.05$, supplementation effect) (Figure 3A, B, C). There was no

570 significant difference between conditions for concentrations of protocatechuic acid, ferulic

571 acid or isoferulic acid (SDC Figure A).

572

573 MCC significantly increased mRNA expression of SOD3, GPX3, GPX4 (supplementation

574 effect $p < 0.05$), GPX7 (supplementation effect $p = 0.001$, interaction effect $p = 0.014$) and

575 GPX1 (interaction effect $p = 0.017$) (SOD3 1.1, GPX3 2.9, GPX4 6.0, GPX7 2.8 and GPX1

576 1.4 fold increase; 2.8 mean fold increase in antioxidant enzyme gene expression with MCC

577 supplementation) (Figure 4). Post-hoc testing revealed significantly greater expression of
578 GPX7 for the MCC condition post-exercise ($p = 0.024$) and at 24-hours ($p = 0.009$) (Figure
579 4H), but no significant differences between conditions at any time points for GPX1 (Figure
580 4E). There was no significant difference between conditions in mRNA expression of SOD1,
581 SOD2, CAT and Nrf2 (Figure 4).
582
583 There was a significant increase in protein expression of GPX3 following MCC
584 supplementation (3.0-fold increase, supplementation effect $p = 0.004$) (Figure 5C). There was
585 no significant difference in SOD1 and CAT protein expression between MCC and PLA
586 (Figure 5A, B). GPX4 and GPX7 proteins were undetectable and are not presented.
587
588 There were significant increases in serum concentrations of IL-6, TNF-α, and CK (time
589 effect $p < 0.05$), but not CRP ($p = 0.130$), following the damage protocol (Figure 6). No
590 significant differences were found between supplementation conditions for serum
591 concentrations of IL-6, TNF-α, CRP and CK (Figure 6). IL-6 data were not normally
592 distributed and could not be normalised by standard approaches to data transformation.
593 However, a non-parametric Friedman's test produced results that were consistent with the
594 results of our 2-way RM ANOVA analysis of these data (time effect $p < 0.001$, supplement
595 effect $p = 0.145$).
596
597 **Discussion**
598
599 This study presents the first evidence demonstrating a significant upregulation of antioxidant
600 gene and protein expression in human skeletal muscle following 7 days pre-load and three
601 days post-load supplementation with MCC containing a complex blend of polyphenols.

602 Crucially, these effects occurred in parallel with significantly improved functional recovery
603 after intensive exercise, as observed previously (9, 10, 13, 15, 16). Furthermore,
604 enhancements in antioxidant expression profile and functional recovery were accompanied
605 by a corresponding augmentation in plasma concentrations of phenolic acids. These novel
606 findings shed new light on the mechanisms that underpin functional changes following
607 natural polyphenol blend supplementation such as MCC.
608
609 Plasma concentrations of 4-hydroxybenzoic acid, hippuric acid and vanillic acid were
610 significantly elevated following MCC supplementation, which demonstrates that
611 supplementation increased circulating exogenous antioxidant concentrations. However, direct
612 ROS scavenging by these compounds is not thought to be the primary mechanism of reduced
613 oxidative damage observed following polyphenol supplementation (18). Indeed, the greatest
614 mean value of plasma phenolic acids measured in the current study did not surpass 40
615 $\mu mol \cdot L^{-1}$ (Vanillic Acid), which is 4-10 fold lower than the values observed for endogenous
616 extracellular antioxidants such as plasma urate, for which concentrations range between 150-
617 450 $\mu mol \cdot L^{-1}$ (7). Although not sufficient to elicit direct antioxidant effects, the increase in
618 plasma concentrations of phenolic compounds appears to have been sufficient to elicit a
619 significant upregulation of endogenous antioxidant gene and protein expression in skeletal
620 muscle in the MCC condition. The mechanism hypothesised to underpin these changes is an
621 upregulation of endogenous antioxidant production via the Nrf2 antioxidant response element
622 pathway following exposure to the aforementioned elevation in phenolic metabolites (19, 20).
623 Nrf2 is widely accepted as the 'master regulator' of antioxidant defence, and upregulation
624 induces an expression profile protective against oxidative stressors through augmented
625 expression of endogenous antioxidants and cytoprotective genes, (34).
626

627 Under normal homeostatic conditions, Nrf2 is repressed through binding to Keap1 within the
628 cytoplasm, where it is ubiquitinated and subsequently proteolysed (35). The proposed
629 activation of Nrf2 following supplementation of MCC is hypothesised to occur via exposure
630 of Keap1 to phenolic metabolites. Indeed, plasma levels of phenolic acids were augmented
631 following MCC supplementation in the present study, supporting previous research which
632 has observed bioavailability of phenolic compounds following consumption of other
633 polyphenol rich supplements (36, 37). These phenolic compounds are then hypothesised to
634 undergo conversion to quinones, semi-quinones and superoxide radicals via dismutation of
635 phenoxyl radicals and redox complexes produced during radical scavenging (38, 39). The
636 literature suggests that cellular exposure to these compounds then causes oxidative
637 modification of Keap1 cystine residues via alkylation (40). Consequently, Nrf2 dissociates
638 from Keap1, enabling nucleic accumulation of Nrf2 and the observed upregulation of
639 endogenous antioxidant gene and protein expression (34, 38, 40).
640
641 Previous *in vitro* and rodent model research has shown evidence that polyphenol exposure
642 can induce Nrf2 gene expression and translocation, as well as augment activity of antioxidant
643 enzymes including GPX and SOD (19, 41), however, to the authors' knowledge, this study
644 presents the first *in vivo* evidence of up-regulation of antioxidant enzyme gene and protein
645 expression in human skeletal muscle following polyphenol supplementation. These novel
646 data provide strong evidence that increased expression of endogenous antioxidant and
647 cytoprotective genes following exposure to phenolic acids, confers protection against
648 oxidative stressors, such as intensive exercise and the resulting inflammatory response;
649 thereby contributing to observed improvements in functional recovery (20, 34) (Figure 7).
650

651 This study is the first to demonstrate an increase in both GPX3 mRNA and protein
652 expression following MCC supplementation. One of the primary postulated mechanisms for
653 reductions in force generating capacity after intensive exercise is altered myofibrillar Ca^{2+}
654 sensitivity (1, 42). Oxidised Troponin I cysteine residues have been shown *in vitro* to bind
655 with glutathione (43). This glutathionylation has a protective effect on Troponin I molecules
656 against oxidative stressors, and increases the Ca2+ sensitivity of the contractile apparatus
657 (41). These mechanisms have in turn been suggested to beneficially affect exercise
658 performance (43). GPX enzymes catalyse the reduction of H_2O_2 and organic hydroperoxides
659 by glutathione and thus their induction (MCC-induced induction of GPX3 in this instance)
660 may contribute to the MCC-induced attenuation of the MVC force reduction identified
661 immediately post-exercise (Figure 2A); this requires considerable further study.
662
663 We describe an ergogenic effect of MCC on recovery of maximal isometric force production
664 in accordance with previous research (9, 10, 13, 15, 16), demonstrating that the
665 aforementioned biological changes occur in parallel with significant functional effects.
666 Importantly, the intensive bout of eccentric exercise induced significant muscle damage as
667 indicated by the significant impairment of all functional measures; measures of perceived
668 soreness; pain pressure threshold at the rectus femoris, and all blood inflammatory markers.
669 Therefore, an experimental paradigm was created in which the favourable effects of MCC
670 supplementation were detectable. Whilst there were no significant differences between
671 supplementation groups for soreness, some of these measures may have been affected by the
672 use of lidocaine local anaesthetic, which, whilst required for the *vastus lateralis* biopsies,
673 may have influenced participants' ability to detect pressure pain. The measure most likely to
674 have been affected in the current study was the PPT measure at the vastus lateralis, as this
675 location was at the closest proximity to the biopsy site. Indeed, there were no significant

676 effects detected for supplement or time point at the vastus lateralis or vastus medialis.
677 However, as there was a significant time effect at the rectus femoris, suggesting that the
678 damage protocol did elicit significant muscle soreness, but pain sensitivity was reduced in the
679 areas closest to the biopsy site. It must also be noted, that a similar phenomenon has been
680 observed previously, where functional enhancements in recovery are not necessarily reflected
681 in soreness or PPT measures (10, 11, 15).
682
683 The lack of difference between conditions in measures of circulating inflammatory cytokines
684 may be due to the methodological limitations associated with the ability of proxy measures
685 from blood to detect subtle changes occurring at the intramuscular level. Indeed, previous
686 research of MCC supplementation that has found significant functional effects have
687 demonstrated equivocal results for blood measures of inflammatory cytokines (9-11, 13).
688 This further highlights the importance of conducting analysis on the exercised tissue, a major
689 limitation of previous research in the area. Unfortunately, due to limited muscle tissue
690 availability, we were unable to characterise inflammatory processes within muscle in the
691 present study. A further limitation in the current study is the lack of quantified dietary intake,
692 without which we cannot be certain of the extent to which background diet, including
693 polyphenol intake, was replicated between trial arms. This may have influenced the observed
694 functional and molecular responses to MCC supplementation. However, visual inspection of
695 dietary logs suggested that intake was faithfully replicated between study arms. Notably, such
696 logs, whether quantified or not are prone to participant reporting errors (44).
697
698 In conclusion, this study showed for the first time that supplementation with US
699 Montmorency cherry concentrate, a polyphenol rich fruit concentrate, significantly increased
700 expression of antioxidant genes and proteins in human skeletal muscle, in parallel with a

701 significant increase in plasma concentrations of phenolic acids. This study also confirmed
702 previous findings that MCC supplementation improved functional muscle recovery from
703 exercise induced muscle damage. This study provides new and compelling evidence to
704 support an upregulation of the antioxidant response element pathway, perhaps due to
705 increased nuclear translocation of Nrf2 following exposure to elevated phenolic metabolites,
706 as the primary mechanism underpinning enhanced functional recovery following polyphenol
707 supplementation.

Additional Information

Data availability statement

Data are available within the figures and tables of this manuscript and are also included in the statistical summary document.

Competing interests

The authors have no competing interests/conflicts of interest to disclose.

Author Contributions

Conception or design of the work (J.T.W., M.F.O., V.G.K., J.L.B.). Acquisition, analysis or interpretation of data for the work (J.T.W., M.F.O., V.G.K., S.R.J., J.D, J.C.Y.T., J.L.B.). Drafting the work or revising it critically for important intellectual content (J.T.W., M.F.O., V.G.K., J.C.Y.T., J.L.B.)

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740
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753
754 The results of the current study are presented clearly, honestly, and without fabrication,
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757 **References**

758

759 1. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and
760 impact on muscle force production. *Physiol Rev*. 2008;88(4):1243-76.
761 2. Reid MB. Redox interventions to increase exercise performance. *The Journal of*
762 *Physiology*. 2016;594(18):5125-33.
763 3. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms.
764 *Physiol Rev*. 2008;88(1):287-332.
765 4. Powers SK, Talbert EE, Adhihetty PJ. Reactive oxygen and nitrogen species as
766 intracellular signals in skeletal muscle. *J Physiol*. 2011;589(Pt 9):2129-38.
767 5. Moopanar TR, Allen DG. Reactive oxygen species reduce myofibrillar Ca2+ sensitivity
768 in fatiguing mouse skeletal muscle at 37 degrees C. *J Physiol*. 2005;564(Pt 1):189-99.
769 6. Smith IJ, Dodd SL. Calpain activation causes a proteasome-dependent increase in
770 protein degradation and inhibits the Akt signalling pathway in rat diaphragm muscle.
771 *Exp Physiol*. 2007;92(3):561-73.
772 7. Bowtell J, Kelly V. Fruit-Derived Polyphenol Supplementation for Athlete Recovery
773 and Performance. *Sports Medicine*. 2019;49:S3-S23.
774 8. Ranchordas MK, Rogerson D, Soltani H, Costello JT. Antioxidants for preventing and
775 reducing muscle soreness after exercise: a Cochrane systematic review. *British*
776 *journal of sports medicine*. 2020;54(2):74.
777 9. Howatson G, McHugh MP, Hill JA et al. Influence of tart cherry juice on indices of
778 recovery following marathon running. *Scandinavian journal of medicine & science in*
779 *sports*. 2010;20(6):843-52.
780 10. Bowtell JL, Sumners DP, Dyer A, Fox P, Mileva KN. Montmorency cherry juice reduces
781 muscle damage caused by intensive strength exercise. *Medicine and science in sports*
782 *and exercise*. 2011;43(8):1544-51.
783 11. Bell PG, Walshe IH, Davison GW, Stevenson EJ, Howatson G. Recovery facilitation
784 with Montmorency cherries following high-intensity, metabolically challenging
785 exercise. *Appl Physiol Nutr Metab*. 2015;40(4):414-23.
786 12. Levers K, Dalton R, Galvan E et al. Effects of powdered Montmorency tart cherry
787 supplementation on acute endurance exercise performance in aerobically trained
788 individuals. *Journal of the International Society of Sports Nutrition*. 2016;13(1):22.
789 13. Bell PG, Stevenson E, Davison GW, Howatson G. The Effects of Montmorency Tart
790 Cherry Concentrate Supplementation on Recovery Following Prolonged, Intermittent
791 Exercise. *Nutrients*. 2016;8(7).
792 14. Gao L, Mazza G. Characterization, Quantitation, and Distribution of Anthocyanins
793 and Colorless Phenolics in Sweet Cherries. *Journal of Agricultural and Food*
794 *Chemistry*. 1995;43(2):343-6.
795 15. Connolly DA, McHugh MP, Padilla-Zakour OI, Carlson L, Sayers SP. Efficacy of a tart
796 cherry juice blend in preventing the symptoms of muscle damage. *British journal of*
797 *sports medicine*. 2006;40(8):679-83; discussion 83.
798 16. Brown MA, Stevenson EJ, Howatson G. Montmorency tart cherry (Prunus cerasus L.)
799 supplementation accelerates recovery from exercise-induced muscle damage in
800 females. *European journal of sport science*. 2018:1-8.

801 17. Jajtner AR, Hoffman JR, Townsend JR et al. The effect of polyphenols on cytokine and
802 granulocyte response to resistance exercise. *Physiological Reports*.
803 2016;4(24):e13058.
804 18. Clifford MN. Diet-derived phenols in plasma and tissues and their implications for
805 health. *Planta Med*. 2004;70(12):1103-14.
806 19. Hwang YP, Choi JH, Yun HJ et al. Anthocyanins from purple sweet potato attenuate
807 dimethylnitrosamine-induced liver injury in rats by inducing Nrf2-mediated
808 antioxidant enzymes and reducing COX-2 and iNOS expression. *Food Chem Toxicol*.
809 2011;49(1):93-9.
810 20. Ji LL, Kang C, Zhang Y. Exercise-induced hormesis and skeletal muscle health. *Free
811 radical biology & medicine*. 2016;98:113-22.
812 21. Abbott W, Brashill C, Brett A, Clifford T. Tart Cherry Juice: No Effect on Muscle
813 Function Loss or Muscle Soreness in Professional Soccer Players After a Match.
814 *International journal of sports physiology and performance*. 2019:1-6.
815 22. Hooper DR, Orange T, Gruber MT, Darakjian AA, Conway KL, Hausenblas HA. Broad
816 Spectrum Polyphenol Supplementation from Tart Cherry Extract on Markers of
817 Recovery from Intense Resistance Exercise. *Journal of the International Society of
818 Sports Nutrition*. 2021;18(1):47.
819 23. Puder JJ, Blum CA, Mueller B, De Geyter C, Dye L, Keller U. Menstrual cycle
820 symptoms are associated with changes in low-grade inflammation. *Eur J Clin Invest*.
821 2006;36(1):58-64.
822 24. Ertel KA, Hallam JE, Hillman AR. The effects of training status and exercise intensity
823 on exercise-induced muscle damage. *J Sports Med Phys Fitness*. 2020;60(3):449-55.
824 25. Bishop PA, Jones E, Woods AK. Recovery from training: a brief review: brief review.
825 *Journal of strength and conditioning research*. 2008;22(3):1015-24.
826 26. McHugh MP. Recent advances in the understanding of the repeated bout effect: the
827 protective effect against muscle damage from a single bout of eccentric exercise.
828 *Scandinavian journal of medicine & science in sports*. 2003;13(2):88-97.
829 27. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical
830 fitness: definitions and distinctions for health-related research. *Public Health Rep*.
831 1985;100(2):126-31.
832 28. Chandra A, Rana J, Li Y. Separation, identification, quantification, and method
833 validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-
834 MS. *J Agric Food Chem*. 2001;49(8):3515-21.
835 29. Shanely RA, Zwetsloot KA, Triplett NT, Meaney MP, Farris GE, Nieman DC. Human
836 skeletal muscle biopsy procedures using the modified Bergstrom technique. *J Vis
837 Exp*. 2014;(91):51812.
838 30. Bijur PE, Silver W, Gallagher EJ. Reliability of the Visual Analog Scale for
839 Measurement of Acute Pain. *Academic Emergency Medicine*. 2001;8(12):1153-7.
840 31. Maeo S, Saito A, Otsuka S, Shan X, Kanehisa H, Kawakami Y. Localization of muscle
841 damage within the quadriceps femoris induced by different types of eccentric
842 exercises. *Scandinavian journal of medicine & science in sports*. 2018;28(1):95-106.
843 32. Marginson V, Eston R. The relationship between torque and joint angle during knee
844 extension in boys and men. *J Sports Sci*. 2001;19(11):875-80.
845 33. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-
846 PCR. *Nucleic Acids Res*. 2001;29(9):e45-e.

847 34. Vomund S, Schafer A, Parnham MJ, Brune B, von Knethen A. Nrf2, the Master
848 Regulator of Anti-Oxidative Responses. *Int J Mol Sci*. 2017;18(12).
849 35. McMahon M, Itoh K, Yamamoto M, Hayes JD. Keap1-dependent proteasomal
850 degradation of transcription factor Nrf2 contributes to the negative regulation of
851 antioxidant response element-driven gene expression. *J Biol Chem*.
852 2003;278(24):21592-600.
853 36. Keane KM, Bell PG, Lodge JK et al. Phytochemical uptake following human
854 consumption of Montmorency tart cherry (L. Prunus cerasus) and influence of
855 phenolic acids on vascular smooth muscle cells in vitro. *Eur J Nutr*. 2016;55(4):1695-
856 705.
857 37. Borges G, Mullen W, Mullan A, Lean MEJ, Roberts SA, Crozier A. Bioavailability of
858 multiple components following acute ingestion of a polyphenol-rich juice drink.
859 *Molecular Nutrition & Food Research*. 2010;54(S2):S268-S77.
860 38. Birringer M. Hormetics: dietary triggers of an adaptive stress response.
861 *Pharmaceutical research*. 2011;28(11):2680-94.
862 39. Eghbaliferiz S, Iranshahi M. Prooxidant Activity of Polyphenols, Flavonoids,
863 Anthocyanins and Carotenoids: Updated Review of Mechanisms and Catalyzing
864 Metals. *Phytotherapy Research*. 2016;30(9):1379-91.
865 40. Satoh T, Kosaka K, Itoh K et al. Carnosic acid, a catechol-type electrophilic
866 compound, protects neurons both in vitro and in vivo through activation of the
867 Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1. *Journal of*
868 *Neurochemistry*. 2008;104(4):1116-31.
869 41. Erlank H, Elmann A, Kohen R, Kanner J. Polyphenols activate Nrf2 in astrocytes via
870 H2O2, semiquinones, and quinones. *Free radical biology & medicine*.
871 2011;51(12):2319-27.
872 42. Steinbacher P, Eckl P. Impact of oxidative stress on exercising skeletal muscle.
873 *Biomolecules*. 2015;5(2):356-77.
874 43. Mollica JP, Dutka TL, Merry TL et al. S-glutathionylation of troponin I (fast) increases
875 contractile apparatus Ca2+ sensitivity in fast-twitch muscle fibres of rats and
876 humans. *The Journal of physiology*. 2012;590(6):1443-63.
877 44. Ravelli MN, Schoeller DA. Traditional Self-Reported Dietary Instruments Are Prone to
878 Inaccuracies and New Approaches Are Needed. *Frontiers in nutrition*. 2020;7:90-.
879

880 **Figure Captions**

881

882 **Figure 1:** Experimental protocol. Montmorency cherry concentrate (MCC), placebo (PLA),
883 warm up (WU), maximal voluntary isometric contraction (MVC), maximal isokinetic
884 contraction (IK^{Max}), single leg jump (SLJ), maximal eccentric contraction (EC^{Max}), Ex
885 (exercise).

886

887 **Figure 2:** Comparison of **A:** maximal voluntary contraction 1s average force **B:** maximal
888 voluntary contraction peak force **C:** peak concentric phase maximal isokinetic contractions
889 **D:** peak eccentric phase maximal isokinetic contractions **E:** maximal single leg jump
890 measured pre-exercise, immediately, 24 hours and 48 hours post exercise for Montmorency
891 Cherry Concentrate (MCC) and Placebo (PLA).*denotes a significantly higher value at that
892 time point for MCC condition vs the placebo condition (Bonferroni Corrected $p<0.05$) or a
893 significantly greater main effect of supplementation for MCC condition vs the placebo
894 condition ($p<0.05$). ‡ denotes a significantly greater interaction effect for MCC condition vs
895 the placebo condition ($p<0.05$). #denotes a significant time effect ($p<0.01^{\#\#}$, $p<0.001^{\#\#\#}$) N =
896 10.

897

898 **Figure 3:** Comparison of plasma phenolic acid concentration measured pre-exercise,
899 immediately and 24 hours post exercise for Montmorency Cherry Concentrate (MCC) and
900 Placebo (PLA). Metabolites displayed are **A:** Hydroxybenzoic Acid **B:** Hippuric Acid **C:**
901 Vanillic Acid. Values are displayed as means±SD. * and **denote a significantly higher
902 value at that time point for MCC condition vs the placebo condition (Bonferroni Corrected
903 *$p<0.05$) or a significantly higher main effect of supplementation for MCC condition vs the
904 placebo condition (*$p<0.05$/**$p<0.01$). ##denotes a significant time effect ($p<0.01$). N = 10

905

906 **Figure 4:** Comparison of antioxidant enzyme gene expression measured pre-exercise,
907 immediately and 24 hours post exercise for Montmorency Cherry Concentrate (MCC) and
908 Placebo (PLA) expressed as **A:** $\log_{10}(x)$ fold change relative to the pre-exercise condition for
909 all measured antioxidant genes, and **B-J:** fold change relative to PLA pre-exercise. **B:**
910 Superoxide Dismutase 1 (SOD1) **C:** SOD2 **D:** SOD3 (N=8) **E:** Glutathione Peroxidase 1

911 (GPX1) **F:** GPX3 **G:** GPX4 **H:** GPX7 (N=8) **I:** Catalase (CAT) **J:** Nuclear Factor Erythroid
912 2–Related Factor 2 (Nrf2). All data are displayed as mean±SD. Data are presented in a
913 manner which allows the reader to visually appreciate both the overall pattern (A – this figure
914 is for visualisation purposes only and does not represent the statistical analysis employed)
915 and actual magnitude (fold-change B-J) of mRNA expression changes. For statistical analysis
916 RTqPCR fold change was calculated using the Pfaffl formula relative to the pre-exercise
917 placebo condition. These values were $\log10(x)$ transformed prior to analysis * and **denote a
918 significantly higher value at that time point for MCC condition vs the placebo condition by 2-
919 way repeated measures analysis of variance (ANOVA) (Bonferroni Corrected
920 *$p<0.05$/**$p<0.01$) or a significantly higher main effect of supplementation for MCC
921 condition vs the placebo condition ($p<0.05$). $^{\#\#}$denotes a significant time effect ($p<0.01$). ‡
922 denotes a significant interaction effect for MCC condition vs the placebo condition ($p<0.05$).
923 N = 9 (unless stated otherwise where values were excluded) (1 participant excluded due to
924 insufficient sample).
925

926 **Figure 5**: Comparison of western blot protein expression for **A:** Superoxide Dismutase 1
927 (SOD1) (N=10) and **B:** Catalase (CAT) (N=10), and **C:** Glutathione Peroxidase 3 (GPX3)
928 (N=9) measured pre-exercise, immediately, 24 hours and 48 hours post exercise for
929 Montmorency Cherry Concentrate (MCC) and Placebo (PLA). All values are presented as
930 mean±SD. * denotes a significantly higher main effect of supplementation for MCC or a
931 significant difference at that time point ($p < 0.05$). **D:** Example blots are displayed for PLA
932 and MCC conditions pre-exercise (Pre), immediately (Post), 24, and 48 hours post exercise.
933 Coomassie stain for protein load is shown for GPX3, stains for SOD1 and CAT are available
934 in supplementary material.

935

936 **Figure 6:** Inflammatory and proxy muscle damage markers in serum **A:** Interleukin 6 (IL-
937 6)(N=9), **B:** Tumour Necrosis Factor Alpha (TNF-α)(N=10), **C:** Creatine Kinase
938 (CK)(N=10), **D:** C-Reactive Protein (CRP)(N=7) measured pre-exercise, immediately, 24
939 hours and 48 hours post exercise for Montmorency Cherry Concentrate (MCC) and Placebo
940 (PLA). All values are presented as mean±SD. $^{\#}$denotes a significant time effect ($p<0.05^{\#}$,
941 $p<0.01^{\#\#}$, $p<0.001^{\#\#\#}$).

942

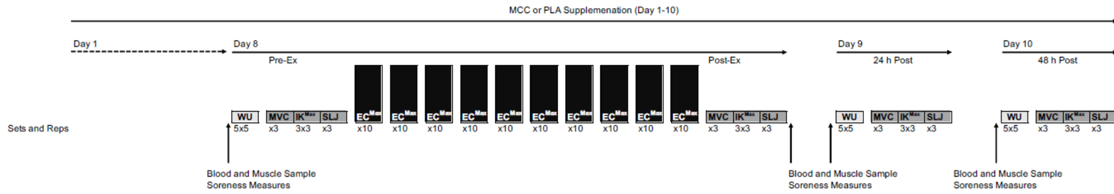
943 **Figure 7:** Hypothesised mechanism underlying increased expression of glutathione
944 peroxidase (GPX) following Montmorency cherry concentrate (MCC) supplementation.
945 Mechanisms demonstrated by the current study are demarcated from hypothesised
946 mechanisms. Kelch-like ECH-associated protein 1 (Keap1), Small musculoaponeurotic
947 fibrosarcoma (sMAF). Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2), Created with
948 BioRender.com

949

950 **Supplemental Digital Content**

951 Supplemental Digital Content. Docx

952

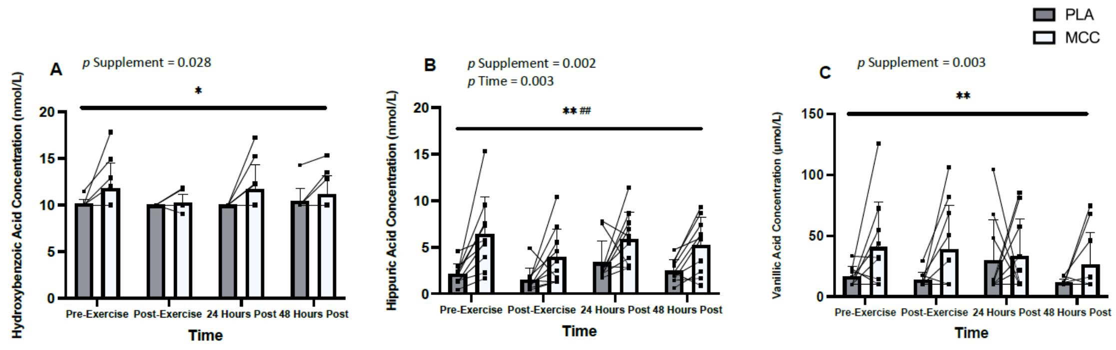


953

954 Figure 1

955

956 Figure 2



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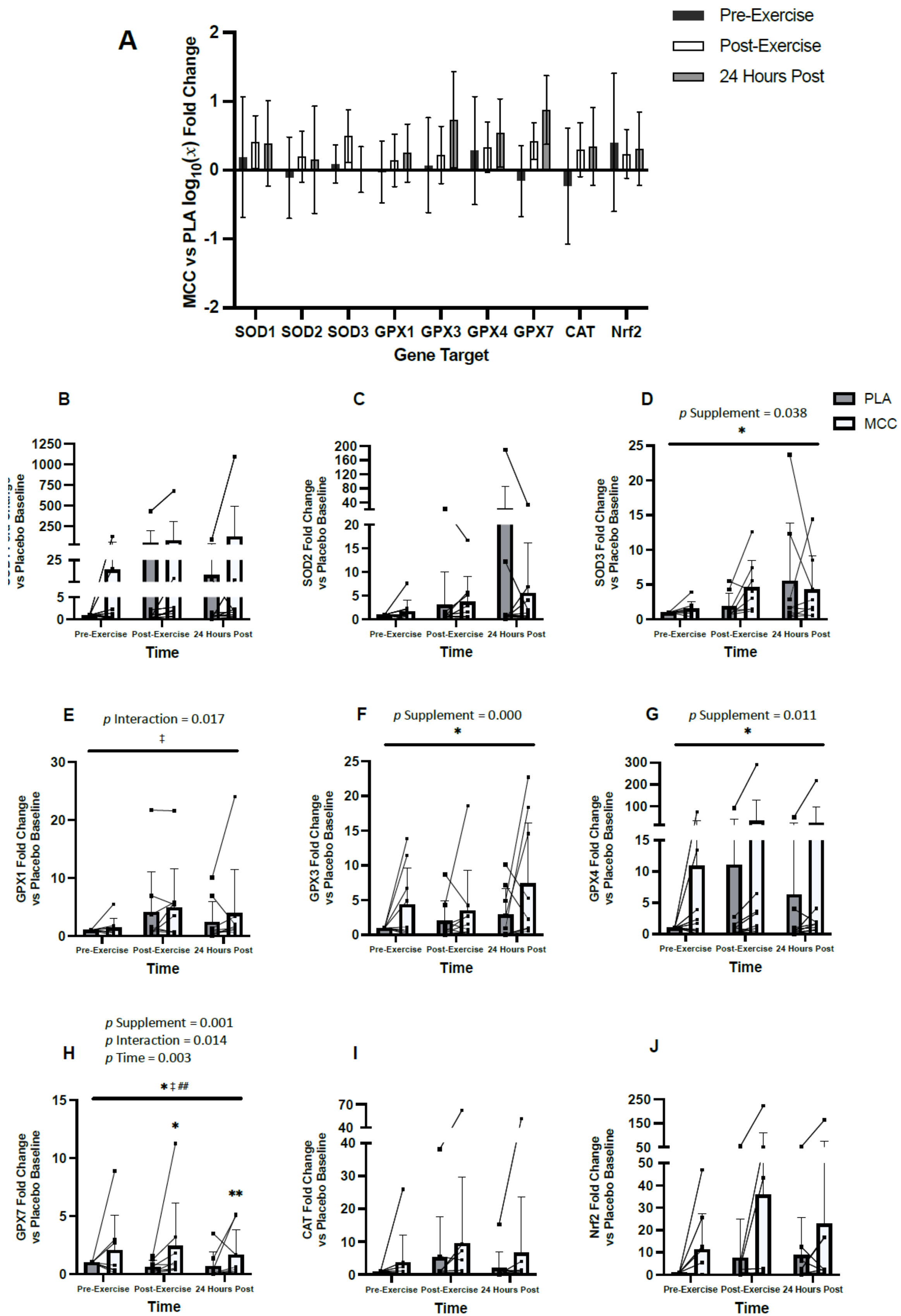
958 Figure 3

959



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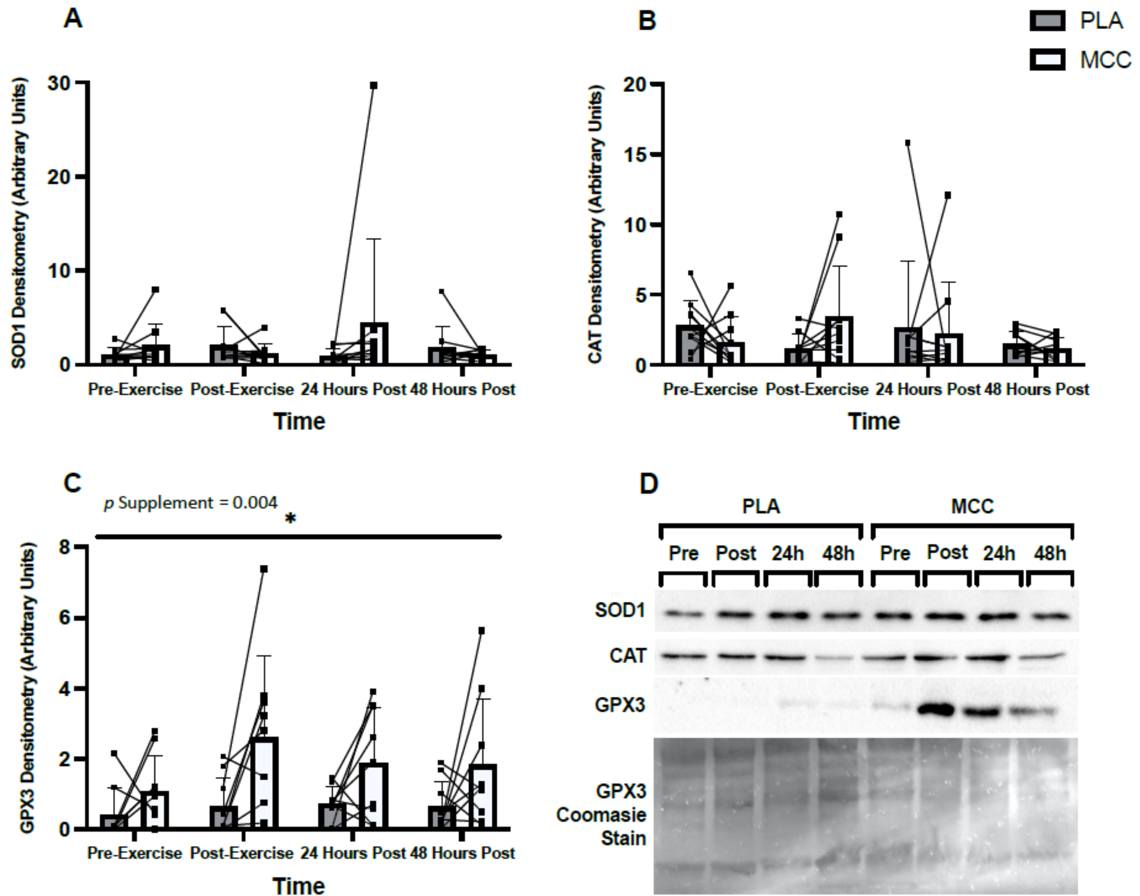
961 Figure 4



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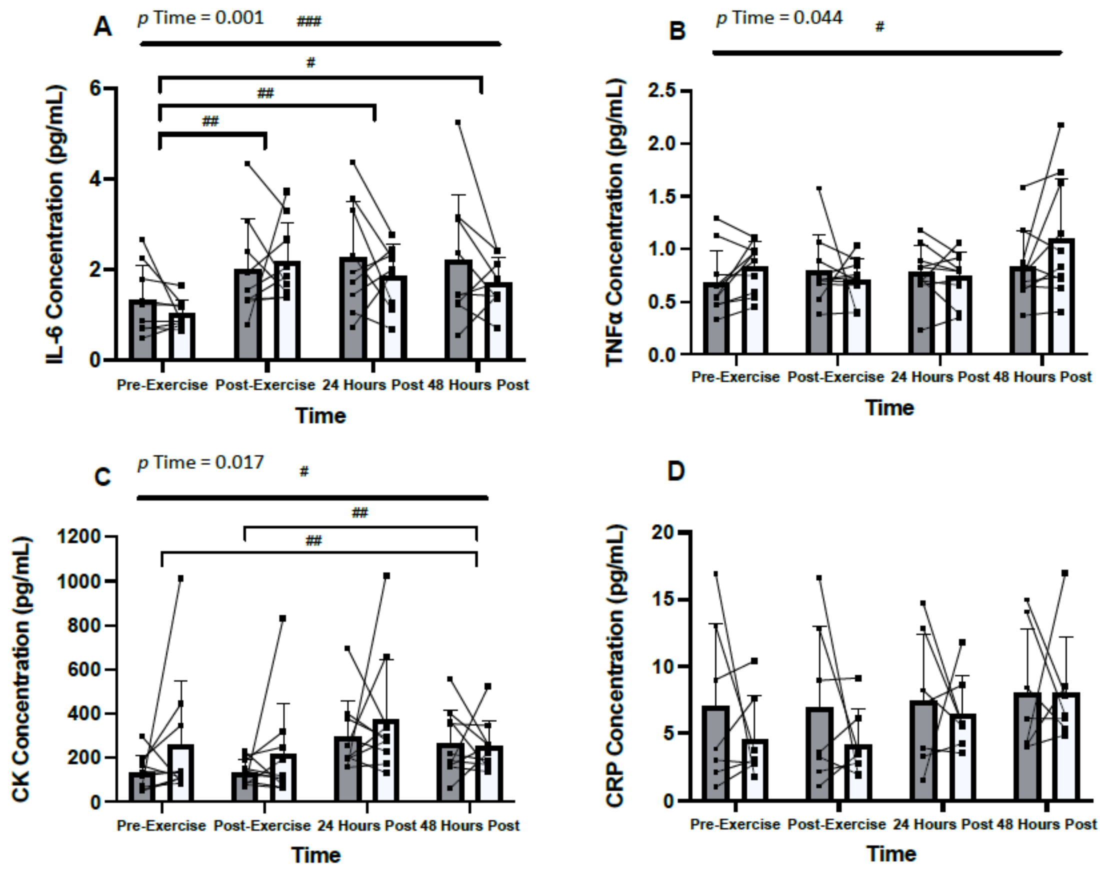
963 Figure 5



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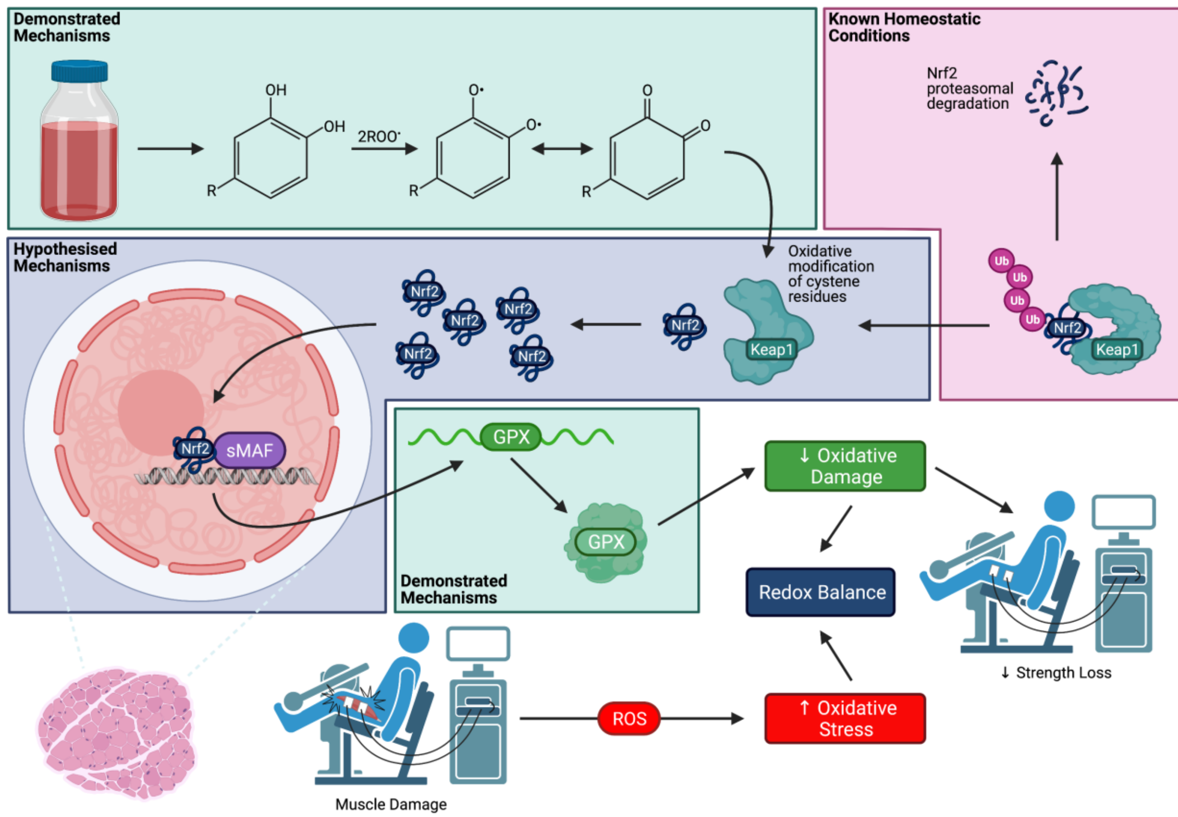
965 Figure 6

966



967

968 Figure 7

969