Tart cherry concentrate does not enhance muscle protein synthesis response to exercise and protein in healthy older men

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Funding:
This work was supported by the Cherry Marketing Institute.

Abstract

Background. Oxidative stress and inflammation may contribute to anabolic resistance in response to protein and exercise in older adults. We investigated whether consumption of montmorency cherry concentrate (MCC) increased anabolic sensitivity to protein ingestion and resistance exercise in healthy older men.

Methods. Sixteen healthy older men were randomised to receive MCC (60ml.d^{-1}) or placebo (PLA) for two weeks, after baseline measures in week 1. During week 3, participants consumed 10g whey protein.d^{-1} and completed three bouts of unilateral leg resistance exercise (4 x 8-10 repetitions at 80% 1RM). Participants consumed a bolus (150mL) and weekly (50mL) doses of deuterated water. Body water ^{2}H enrichment was measured in saliva and vastus lateralis biopsies were taken from the non-exercised leg after weeks 1, 2 and 3, and the exercised leg after week 3, to measure tracer incorporation at rest, in response to protein and protein+exercise.

Results. Myofibrillar protein synthesis increased in response to exercise+protein compared to rest ($p<0.05$) in both groups, but there was no added effect of supplement (MCC: 1.79±0.75 EX vs 1.15±0.40 rest; PLA: 2.22±0.54 vs 1.21±0.18; all %.d^{-1}). Muscle total NFκB protein was decreased with exercise and protein in MCC (NFκB: -20.7±17.5 %) but increased in PLA (NFκB: 17.8±31.3 %, $p=0.073$).

Conclusion. Short-term MCC ingestion does not affect the anabolic response to protein and exercise in healthy, relatively active, older men, despite MCC ingestion attenuating expression of proteins involved in the muscle inflammatory response to exercise, which may influence the chronic training response.

Keywords: polyphenols, inflammation, deuterated water, myofibrillar, montmorency.

Introduction

Progressive age-related loss of muscle mass (sarcopenia) commences in the 4/5th decade and accelerates from the 6th decade onwards with per annum losses in the order of 0.5 – 1.5% [1], and ~ 1.5 % per annum strength losses [2]. These declines lead to a loss of independence and impaired quality of life in otherwise healthy older individuals [2], so interventions that limit age-related loss of muscle mass and function are urgently required.

The response of muscle protein synthesis (MPS) and breakdown (MPB) to nutritional intake and activity levels maintain muscle mass in healthy adults. Decreases in muscle mass occur as a consequence of MPB chronically exceeding MPS. Basal rates of MPS [3] and MPB [4] do not seem to be affected by age *per se* in healthy men. Small elevations in MPB after resistance exercise (RE) related to the activation of the ubiquitin proteasome and autophagal lysosomal systems are not augmented in older people [5]. However it is clear that older subjects display resistance to anabolic signals such as provision of essential amino acids [3] and RE [6], resulting in attenuated MPS responses compared to their younger counterparts, which is pivotal to the progression of sarcopenia. The mechanisms underlying this blunted response to feeding and exercise are still unclear, but are likely associated with age-related declines in habitual activity [7; 8], and low level but chronic oxidative stress and inflammation.

Oxidative stress related carbonylation of muscle proteins has been associated with the age-related muscle mass loss in rodents [9]. Markers of oxidative damage are also elevated in muscle tissue biopsied from elderly men, with mitochondrial protein carbonyl adducts more abundant in muscle of old versus young men and associated with reduced muscle strength [10]. The inflammatory pathway is also implicated in age-related anabolic resistance. TNFα seems to impair MPS via decreased phosphorylation of proteins in the mTOR signalling pathway [11], that is critical for regulation of mRNA translation and the MPS increases required for muscle hypertrophy [12]. In elderly muscle we found a 4-fold elevation in NF-κB concentration, which was associated with a reduction in concentration and phosphorylation of key components of the muscle protein synthesis response (mTOR, p70^{s6}k and eIF4BP-1) to AA provision [3].

Control of this underlying inflammation would appear to be an appropriate target for therapeutic intervention. However, acute anabolic effects of RE seem to be, at least in part, mediated through a brief induction of NF-κB signalling and downstream inflammatory pathway gene expression (IL-6, IL-8, MCP-1) [13]. Interestingly non-steroidal anti-inflammatory agents (NSAIDs) that inhibit cyclo-

oxygenase activity (COX 1 and 2) exert opposite effects in young and elderly muscle: blocking the normal anabolic response to acute resistance exercise in young men [14], reducing the hypertrophy associated with an 8 week resistance training programme [15], in parallel with abolition of the usual increase in the anabolic agent prostaglandin $F_{2\alpha}$, a COX product [16]. In contrast daily consumption of acetaminophen or ibuprofen during a 12 week resistance training programme augmented (~25-50%) muscle hypertrophy and strength gains in elderly individuals [17]. Although muscle protein turnover data are not available, Trappe et al [18] demonstrated that NSAID consumption in parallel with a resistance training programme upregulated PGF2α receptors, and reduced intramuscular PGE2 production in the elderly. We propose therefore that this discrepancy in NSAID effects in the young and old, whereby anabolic sensitivity to exercise is restored in the old but not the young, by counteracting the low level chronic systemic inflammation present in the elderly. Certainly NSAIDs were not able to attenuate atrophy induced 2 weeks of immobilisation or enhance hypertrophy during 2 weeks of retraining in healthy older adults with low plasma C reactive protein concentration [19].

The known side effects associated with chronic NSAIDs supplementation, including gastrointestinal injury [20], would prevent the chronic application of this prophylactic approach to ameliorate sarcopenia. However, there is increasing interest in the application of nutritional supplements with known anti-inflammatory effects such as omega-3 fatty acids, which restored anabolic sensitivity to amino acids and hyperinsulinaemia in older individuals [21]. Tart cherries are rich in polyphenols, the anthocyanins and proanthocyanidins in particular [22; 23]. *In vitro* cherries are potent anti-oxidants [22; 23] and inhibit COX1 [22; 23] and COX2 [23] activity. In mice, tart cherry juice consumption increased hepatic superoxide dismutase and glutathione peroxidase activity and inhibited COX2 activity [24], possibly mediated by activation of the nuclear erythroid 2-related factor 2 (Nrf2) pathway resulting in increased synthesis of endogenous antioxidants [25]. A number of studies including our own have demonstrated that montmorency cherry juice improves functional recovery from a single bout of intensive resistance [26] or endurance exercise [27], with associated reductions in circulating biomarkers of oxidative damage (protein carbonyls) and inflammation (C reactive protein). We hypothesised that this cherry-induced reduction in oxidative stress and inflammation will restore anabolic sensitivity in older adults. Our aim was to determine whether two weeks of daily montmorency cherry concentrate (MCC) supplementation enhanced the anabolic response to protein supplementation and resistance exercise in healthy older men, measured as free-living myofibrillar protein synthesis using the deuterated water technique [28].

Methods

Participants

The study was approved by the local university ethics committee, and was conducted in compliance with the World Medical Association's Declaration of Helsinki (2008). We screened and recruited sixteen healthy older men aged 60-75y, all of whom gave their written informed consent to participate. Exclusion criteria included allergy to local anaesthetic or iodine, reporting more than 150 min moderate/vigorous activity per week (in addition to tasks of daily living), consumption of anti-inflammatory medication or nutritional supplements, neuromuscular problems or acute knee/ ankle injuries and pain.

Experimental design

Participants completed a three week experimental period in this double blind randomized control trial, which included four laboratory visits. During the first week participants were instructed to maintain habitual activity and diet. During week 2 and 3 participants consumed 30 mL of either Montmorency cherry concentrate (MCC) or cherry flavoured isoenergetic placebo (PLA) twice per day (morning and evening); diluted with water 5:1. Additionally participants consumed 10g of protein each day in week 3 and completed three unilateral resistance training sessions (Figure 1). MCC provided 40g CHO, 192 kcal and 540 mg anthocyanins per day (HPLC analysis, Atlas Bioscience Inc). PLA contained cherry flavoured cordial (Blossom Cottage, Bottle green) and sugar to match CHO (40g) and energy content.

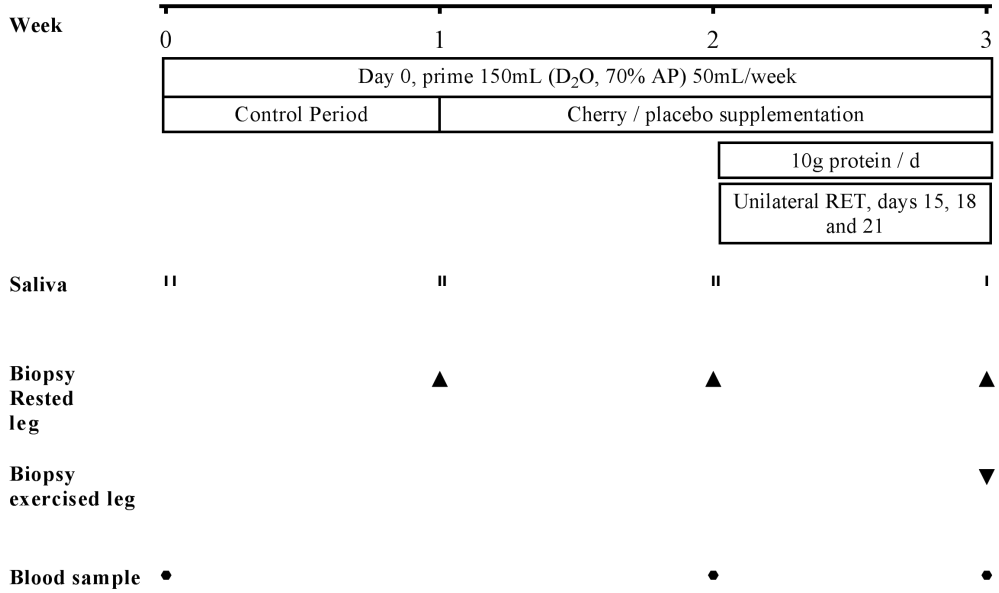

Figure 1: Experimental protocol. RET = resistance exercise training, each session consisted of four sets of 8-10 repetitions at 80% one repetition maximum

Experimental protocol

At the first laboratory visit, participants gave baseline saliva and blood samples, after which they received three 50 mL doses of D_2O separated by at least 30 min. Participants completed a physical activity questionnaire (IPAQ), and body weight and height were measured, and the single leg knee extension one repetition maximum (1RM) was estimated using a 10 repetition maximum (10RM) protocol whereby 1RM was calculated using the weight lifted/ (1.0278-0.0278* number of completed repetitions) [29]. The exercising leg was counterbalanced for leg dominance across participants and supplement group. After this participants departed with instructions to provide a further saliva sample 3 h after consuming the last D_2O dose. This was stored in the fridge and returned at the next visit one week later. At the second visit, after collecting a saliva and blood sample, the first muscle biopsy (PRE-SUP) was taken from the vastus lateralis muscle of the non-exercising leg. A further 50 ml dose of D_2O was consumed, and a saliva sample collected 3 h later. Participants were provided with 4 x 210 mL bottles containing either an isoenergetic placebo (PLA) or CherryActive concentrate (MCC) and instructed to consume 30 mL every morning and evening for the following 2 weeks. One week later participants returned to the laboratory for saliva, blood and muscle sample from the control leg (SUP), and a final 50 ml dose of D_2O. After this participants completed the first single leg knee extension exercise training session (see below), and consumed 10g whey protein (MyProtein). A saliva sample was collected 3 h after the final D_2O dose. Participants completed two further training sessions each separated by 48 h. Participants consumed 10g whey protein every day between breakfast and lunch on non-training days and immediately post training on training days. Twenty-four hours after the last training session, participants returned for a final laboratory visit, at which saliva and blood samples were collected and muscle biopsies were taken from the rested (SUP+PRO) and exercised (SUP+PRO+Rex) leg.

Muscle, saliva and blood collection

Muscle biopsies were obtained from the vastus lateralis under local anaesthesia (2 % lidocaine) using 5 mm Bergstrom needles with suction, frozen in liquid nitrogen, and stored at -80 °C until later analysis. Supplements were consumed after biopsy collection on test visits to avoid contraindications. Saliva samples were collected > 30 min post food or drink ingestion at each laboratory visit and at 3 h post ingestion of each D_2O dose. Samples were centrifuged at 4000 rpm for 10 min at 4 ^{O}C and the supernatant stored in eppendorfs at -80 °C. Blood samples were collected via venipuncture at each laboratory visit and aliquots of plasma and serum were then frozen at -80 °C until later analysis for serum IL6 and protein carbonyls using a commercially available assay kit (IDS and Biocell

respectively). Plasma hsCRP concentration was measured via immunoturbidometric method using a Roche P800 analyser (Roche 800, Mannheim, Germany).

Diet and Physical activity

Participants completed three day weighed food diaries during weeks 2 and 3, and diaries were analysed using Compeat nutritional analysis software (Nutrition Systems, Banbury, UK). Physical activity was assessed using GeneActiv accelerometers (ActivInsights Ltd., Kimbolton, Cambridgeshire, United Kingdom), placed on the left wrist of participants after collection of the second biopsy and worn continuously for the next seven days. Triaxial acceleration was measured between ±8g, at 100Hz. At the end of the data collection period, data were downloaded to a personal computer according to manufacturer's instructions and raw triaxial acceleration values were converted to resultant acceleration. Vector magnitude (VM) was taken from the three axes and then subtracted by the value of gravity (g) as in $(x^2 + y^2 + z^2)^{½} – 1$ [30]. Data were then averaged over 60s-epochs and previously validated acceleration cutpoints were then applied to identify sedentary time and time spent performing light, moderate and vigorous physical activity over the one week period [30].

Resistance exercise protocol

The participants completed the single leg resistance exercise (leg press) on three occasions. On each occasion the participants completed a standardised warm-up which consisted of 12 repetitions at 40% 1RM, 10 repetitions at 50% 1RM, 8 repetitions at 60% 1RM and 2 repetitions at 70% 1RM Participants then completed four sets of 8-10 repetitions at 80% 1RM separated by 2 min. When participants could not lift the weight and complete 10 repetitions the weight was subsequently lowered by 2.5kg for the next set, weight was never increased.

Muscle Protein Synthesis

Body water enrichment was determined through direct liquid injection of saliva samples (0.1µl volume) into a High Temperature Conversion Elemental Analyser (TC/EA; Thermo Finnigan, Thermo Scientific, Hemel Hempstead, UK) connected to an Isotope Ratio Mass Spectrometer (IRMS, Delta V Advantage, Thermo, UK). Myofibrillar and sarcoplasmic protein fractions were isolated from ~30-50 mg of muscle as previously described (22). [28] Myofibrillar protein derived AA were derivatized as their n-methoxycarbonyl methyl esters (MCME) [28] and incorporation of deuterium into protein bound alanine was determined by gas chromatography-pyrolysis isotope ratio mass spectrometry (Delta V Advantage, Thermo Scientific, Hemel Hempstead, UK), alongside a standard

curve of known L-Alanine-2,3,3,3-d4 enrichment. The deuterium isotopic enrichment provided as δ^2H was converted to atom % using the following equation:

$$\text{Atom\%} = \frac{100 \times AR\ (d^2H \times 0.001 + 1)}{1 + AR\ (d^2H \times 0.001 + 1)}$$

Where, AR represents the absolute ratio constant for deuterium based on the VSMOW standard and equates to 0.00015595. This was then converted to atom % excess (APE) by correcting for baseline sample i.e. background enrichment.

The fractional synthetic rate (FSR) of myofibrillar (MyoPS) protein synthesis was determined from the incorporation of deuterium labelled alanine into protein, using the enrichment of body water (corrected for the mean number of deuterium moieties incorporated per alanine, 3.7) as the surrogate precursor labelling between subsequent biopsies. In brief, the standard equation:

$$FSR = -Ln\left(\frac{1 - \left[\frac{(APEala)}{(APEp)}\right]}{t}\right)$$

Where, APEala = deuterium enrichment of protein bound alanine, APEp = mean precursor enrichment over the time period and *t* is the time between biopsies.

Muscle Protein and Gene Expression

Muscle lysate was extracted by homogenizing muscle in 10ul/mg of ice-cold homogenization buffer [50 mM Tris·HCl (pH 7.4), 50mMNaF, 10mM β-glycerophosphate disodium salt, 1 mM EDTA, 1 mM EGTA, and 1 mM activated Na_3VO_4 (all from Sigma-Aldrich)] and a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK). Protein concentrations were determined by spectrophotometry (NanoDrop Lite); with samples standardized to 1 mg ml^{-1} by dilution with 3× Laemmli loading buffer and heated at 95°C for 5 min. Sarcoplasmic protein fractions were analyzed as described previously[31], with four biopsies for each participant analysed on the same gel with each gel containing participants from both experimental groups to control for intra-assay variability. Briefly ~15µg/well of protein was separated on 4–20% gradient gels (BioRad Criterion; Bio-Rad Laboratories), using XT mops running buffer (Biorad) and transferred to PVDF membranes (Immobilon-P; Millipore, Bedford, MA). After blocking, membranes were incubated with appropriate antibodies diluted 1:200 at 4°C overnight: phospho-NFκB S536 (Cell Signaling Technology 93H1), total NFκB (Cell Signaling Technology), total IκκB (Cell Signaling Technology), TNF-α (Cell Signaling Technology), and COX-1 (Abcam). Membranes were subsequently incubated

with secondary antibodies and exposed with enhanced chemiluminescent substrate (Millipore) captured using a chemiluminescent imaging system (Chemidoc, Bio-rad), with sizes of the immunodetected proteins confirmed by molecular weight markers (ladder, Invitrogen). Blots were stripped in stripping buffer (ThermoFisher) for 5-10 minutes at room temperature. Membranes were then re blocked, incubated with secondary antibody and exposed as above to test for sufficient antibody removal, with stripping performed no more than once when required. Equal protein loading was verified by Coomassie staining, shown to be preferential loading control as discussed in [32].

Briefly, total RNA was isolated from 5-10 mg of muscle using TRizol (LifeTechnologies, ThermoFisher Scientific) and resuspended in 22 µl of RNase-free water and quantified by spectrophotometry (NanoDrop Lite). For RT-qPCR 500 ng of total RNA was reversed-transcribed with the high-capacity cDNA reverse transcription kit (Life Technologies) according to manufacturer's protocol. The resulting cDNA was diluted 1:5 and 1 µl was added per well of the 384-optical well plates (Life Technologies). Exon specific primers were mixed with SYBR Select Master Mix (Life Technologies) and 11 µl of master-mix added to each well, with each sample run in triplicate on a ViiATM 7 Real-Time PCR System (Life Technologies). To control for RNA input, PPIA levels were measured and target mRNA expression was quantified using the ΔΔCt method [33].

Statistics

Participant characteristics were analysed using independent samples t-test. Data were analysed by two way mixed model ANOVA condition (cherry, placebo) vs time (basal, SUP, SUP+PRO, SUP+PRO+EX) to determine whether MCC and PLA differentially affected: resting measures, protein response, and protein and exercise response. Post hoc analysis was completed to identify the specific location of differences with Bonferroni correction for multiple comparisons. Statistical significance was accepted at $p<0.05$. Analysis was performed using SPSS version 22 (IBM, Chicago, Illinois, USA), data are presented as mean±SD unless otherwise stated.

Tissue was not obtained from one participant at baseline and for another participant after week 1 of supplementation therefore data is expressed as n= 8 in PLA and n=6 in SUP. In all other measures n=8 in SUP and PLA.

Results

Participant characteristics and physical activity

All measured anthropometric characteristics, knee extensor strength, and training load did not differ between groups (Table 1). There was no difference between groups in either the amount of time spent completing moderate and vigorous physical activity or sedentary time (Table 1). Nor was there any significant difference in the energy or protein intake between groups in either week 2 or 3 of the protocol. However, protein intake was significantly higher in both groups in week 3 than 2 with the daily consumption of 10g whey protein (p=0.004, Table 2).

Table 1. Anthropometric and training data

	MCC	**PLA**
Age (y)		
Weight (kg)	83.8 ± 13.9	76.1 ± 9.3
Height (m)	1.76 ± 0.12	1.77 ± 0.05
BMI ($kg.m^{-2}$)	26.4 ±1.7	24.4 ± 2.7
1RM (kg)	36.1 ± 8.2	37.9 ± 10.2
Training load during exercise week (kg)	1699 ± 379	1798 ± 478
MVPA ($min \cdot d^{-1}$)	117 ± 55	153 ± 69
Sedentary time ($min \cdot d^{-1}$)	429 ± 130	396 ± 208

Means ± SD for participants in the cherry (MCC) and placebo (PLA). 1RM = one repetition maximum. MVPA = Moderate and vigorous physical activity

Table 2. Nutritional analysis data.

	Week 2		**Week 3**	
Group	**Energy Intake ($kcal.d^{-1}$)**	**Protein intake ($g.kgBM.d^{-1}$)**	**Energy Intake ($kcal.d^{-1}$)**	**Protein intake ($g.\ kgBM.d^{-1}$)***
MCC	1462 ± 206	0.79 ± 0.10	1560 ± 494	0.99 ± 0.25
PLA	1967 ± 375	1.05 ± 0.32	1944 ± 359	1.22 ± 0.23

Means ± SD for participants in the cherry (MCC) and placebo (PLA) groups during weeks 2 and 3 of the protocol, n=8 per group, * - significantly different to week 2, $p<0.05$

Saliva enrichment

Saliva ^{2}H enrichment changed significantly over time (main effect of time $p < 0.01$, Figure 2) but these differences were not affected by condition.

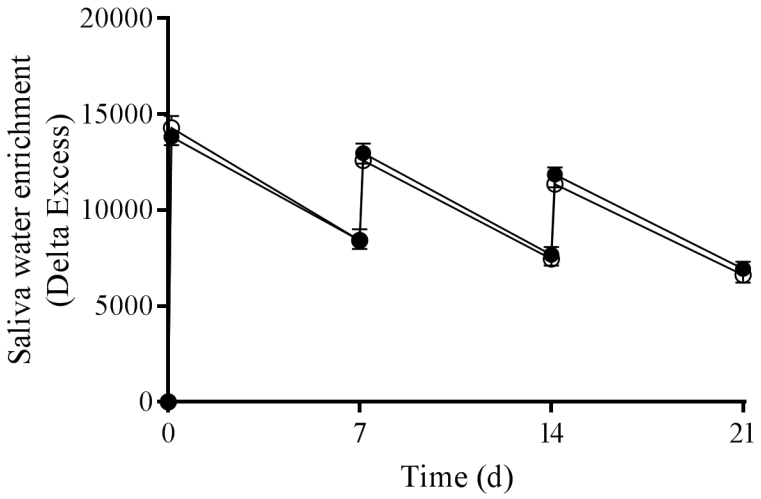

Figure 2: Time course of saliva enrichment (Delta Excess) throughout the experimental period. Data is shown pre (0, 7d) and following (14, 21d) supplementation of either montmorency cherry juice (MCC, closed circles) or a placebo (PLA, open circles).

Myofibrillar protein synthesis

Myofibrillar protein synthesis changed significantly across the experimental period (main effect of timepoint, $p<0.001$), with MPS significantly higher after exercise+protein compared to all other time points ($p<0.05$, Figure 3). However, in the non-exercised leg protein alone did not increase MPS, nor was the increase in MPS induced by exercise and protein significantly different between supplements (22±19 %, MCC; 39±11 %, PLA; $p=0.4$).

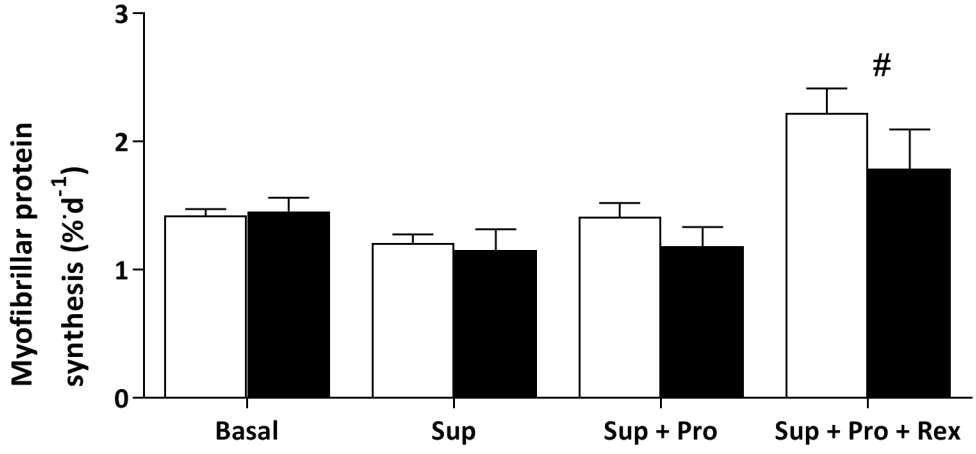

Figure 3: Myofibrillar protein synthesis at baseline and in response to one week of supplementation (SUP) either with Montmorency cherry extract (MCC; black bars) or placebo (white bars), two weeks of supplementation and consuming 10g protein per day for one week in the rested leg (SUP+PRO), and the exercised leg (SUP+PRO+EX). Data are presented as mean±SE, n=8 in PLA and n=6 in SUP. # time effect compared to all other timepoints

Muscle Protein Expression

All western data are presented relative to Coomassie density. Total NFκB (Figure 4a) tended to increase in response to exercise and protein in the PLA condition (+18%) but to decline in the MCC condition (-21%) ($p=0.073$, main interaction effect). Phosphorylated-NFκB (Figure 4b) increased significantly after protein and exercise ($p=0.023$, main time effect), but these changes were not affected by condition. Neither muscle TNFα nor COX1 protein expression changed in response to exercise or protein supplementation in either condition (table 3).

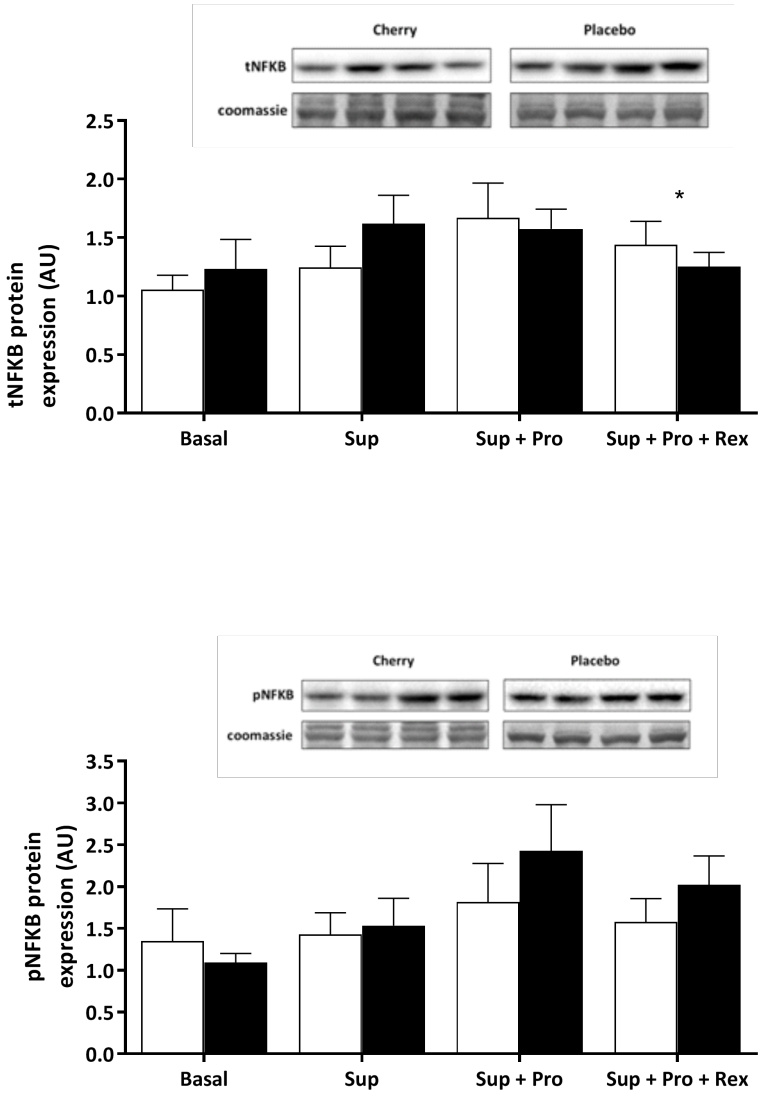

Figure 4 Expression of A – total NFκB protein and B - pNFκB protein in the sarcoplasmic muscle fraction at baseline and in response to one week of supplementation (SUP) either with Montmorency cherry extract (MCC; black bars) or placebo (white bars), two weeks of supplementation and consuming 10g protein per day for one week in the rested leg (SUP+PRO), and the exercised leg (SUP+PRO+EX). Data are presented as mean±SE, n=8 for PLA, and n=7 for MCC except basal where n=6; * $p<0.05$ main interaction effect versus SUP.

Table 3: Muscle protein and gene expression and serum markers of inflammation

Parameter		Basal	SUP	SUP+PRO	SUP+PRO+EX
COX 1 protein	**MCC**	0.79 ± 0.21	1.07 ± 0.43	1.02 ± 0.70	0.83 ± 0.24
(AU)	**PLA**	0.73±0.28	0.72 ± 0.27	0.74 ± 0.29	0.74 ± 0.22
TNFα protein	**MCC**	0.71± 0.26	0.72 ± 0.36	0.74 ± 0.53	0.66 ± 0.47
(AU)	**PLA**	0.80± 0.63	0.88 ± 0.75	0.92 ± 0.70	0.94 ± 0.58
COX 1 gene	**MCC**	0.05± 0.03	0.04 ± 0.02	0.05 ± 0.04	0.07 ± 0.04
expression (AU)	**PLA**	0.07± 0.05	0.10 ± 0.08	0.07 ± 0.02	0.09 ± 0.07
TNFα gene	**MCC**	0.33± 0.35	0.22 ± 0.04	0.13 ± 0.04	0.26 ± 0.24
expression (AU)	**PLA**	0.25± 0.14	0.22 ± 0.07	0.24 ± 0.14	0.24 ± 0.08
NFκB gene	**MCC**	1.46±1.02	1.26 ± 0.58	0.87 ± 0.36	1.54 ± 0.89
expression (AU)	**PLA**	1.17 ±0.40	1.21 ± 0.60	0.97 ± 0.40	1.21 ± 0.64
IL1B gene	**MCC**	0.27 ± 0.18	0.20 ± 0.04	0.13 ± 0.06	0.21 ± 0.04
expression (AU)	**PLA**	0.22 ± 0.13	0.16 ± 0.05	0.20 ± 0.11	0.20 ± 0.08
NrF2 gene	**MCC**	1.91 ± 1.00	2.21 ± 1.10	1.96 ± 0.75	3.34 ± 2.82
expression (AU)	**PLA**	1.93 ± 0.74	1.68 ± 0.58	2.32 ± 1.00	1.98 ± 0.84
Serum IL6	**MCC**	1.58 ± 0.78	1.84 ± 1.46	1.61 ± 1.39	
	PLA	1.29 ± 0.98	0.92 ± 0.49	1.03 ± 0.90	
Plasma CRP	**MCC**	2.24 ± 1.48	1.64 ± 0.84	2.12 ±2.01	
	PLA	2.34 ± 3.36	1.41 ± 1.38	0.68 ±0.71	
Serum protein	**MCC**	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	
carbonyls	**PLA**	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	

Means ± SD for participants in the cherry (MCC) and placebo (PLA) groups.

Muscle Gene Expression

There were no effects of supplement, protein, or protein + exercise on expression of Nrf2, TNFα, COX1, NFκB or IL1β genes (table 3).

Plasma Analytes

Protein carbonyls, CRP and IL6 were not different at any time point and there was no effect of supplement ($p>0.05$) (table 3).

Discussion

Short term consumption of montmorency cherry concentrate providing ~540 mg anthocyanins.d^{-1} in healthy, relatively active, older men did not influence myofibrillar protein synthesis rates. However, the exercise-induced changes in NFκB protein expression in muscle tended to be attenuated by MCC consumption. The effect of such inflammatory pathway changes on the adaptive response to exercise, require further investigation. Our data demonstrate that three resistance training sessions with consumption of additional protein (10g.d^{-1}) augmented the response of myofibrillar protein synthesis over a one-week free-living period in healthy older men. However consumption of additional protein (10g.d^{-1}) on its own was not sufficient to induce an anabolic response.

In response to a bout of RE, MPS is acutely (60-90 mins) increased in healthy young and older men [6] and these effects may last for up to 48h after exercise, at least in young men [34]. This response is augmented when combined with protein intake both in young [35] and older adults [6]; therefore repeated bouts of RE with supportive nutrition should ultimately lead to muscle hypertrophy. D_2O enables longer-term integrated measures of MPS that captures these combined responses over days and weeks. Using this technique we have previously shown heightened MPS in young individuals of ~ 1.8%.d^{-1} over eight days of RE training [28] and this elevated MPS with RE is sustained for ~ three weeks. The magnitude of both acute and integrative measures of MPS decline thereafter, despite continued progression of exercise intensity [36]. Here we show that in response to three bouts of RE and daily protein supplementation integrated MPS is increased to ~1.7-2%.d^{-1} over seven days in healthy older men, similar to values we have previously reported in young subjects over this time [28]. However, there was no effect of MCC supplementation.

There are limited comparative data for integrative MPS measures in older adults. Recently, MPS was shown to be increased over 3d with one bout of RE (3 sets to failure at 50% 1RM, ~15-24 reps) from 1.48%.d^{-1} to ~1.71%.d^{-1}. This effect was augmented to 1.87%.d^{-1} by the consumption of additional dietary leucine (5 g per meal to achieve 15g.d^{-1}) [37]. In support of this data, our participants completed three bouts of RE (four sets of 8-10 reps at 80 % 1RM) and consumed an additional 10g of whey protein (~1.6g Leu) per day over the 7d period and achieved higher rates of MPS 2.0%.d^{-1}, suggestive of a sustained effect. In common with these studies our data indicate that an acute MPS response (< 1week) to RE occurs in older adults. However, the role this increase has on muscle homeostasis and contributions to muscle hypertrophy are unclear. For instance if these rates of MPS were maintained in the absence of changes in MPB, considerable hypertrophy would be expected, when in fact RE training-induced hypertrophy is often attenuated in older versus young participants [38]. In addition, we have recently shown in young and older individuals undertaking the same RE training (at least in terms of relative intensity) that old subjects showed blunted MPS over three weeks and this is

reflected with impaired muscle hypertrophy [39]. The initial higher rates of MPS, observed over 2d [40], 3d [37] and 7d in this study may simply be the result of substantial repair processes that occur in response to bouts of RE to which participants were relatively unaccustomed. Ultimately the magnitude of response may subside as responses become more 'hypertrophy specific' with continued RE training [41]. In support of this, well-trained master athletes, displayed MPS rates of ~ 1.4%.d^{-1} over 3 d in response to intense endurance exercise, compared with 1.7%.d^{-1} in young triathletes [42].

We chose a lower dose of protein intentionally, to elicit a sub-maximal anabolic response such that there was still capacity to reveal any additional anabolic effects of MCC. Our participants were relatively active completing 135 (± 60) min of objectively measured moderate-to-vigorous activity per day (equivalent to 14.7 % of waking hours) in this study - close to the FAO/WHO/UNU recommendation of 15% of waking hours in MVPA [43]. It is important to note that the MPS measures incorporated the integrated effects of habitual activity and dietary behaviour throughout the week. A small effect of MCC on MPS could therefore be masked by small changes in either physical activity levels and/or protein intake, especially in view of the relatively small sample size for MPS due to the loss of muscle tissue. Habitual protein intake was also sufficiently low (<1 g.kg^{-1} BM.d^{-1}) to potentially hamper optimal MPS response. The effect that habitual protein intake and activity levels, individually or in combination, have on integrated measures of MPS is unknown and requires further study.

We hypothesised that MCC would enhance anabolic responses in older men by attenuating oxidative damage and inflammation. However, the healthy older male participants in this study did not exhibit elevated systemic markers of inflammation (CRP and IL6); nor was there any elevation in protein carbonyls (a marker of oxidative modification of protein). These blood data suggest that the study population were not experiencing the systemic oxidative stress and inflammation suggested to contribute to age-related anabolic resistance. It is perhaps unsurprising, therefore, that in these circumstances no effects of MCC on MPS response to exercise and protein were observed, and future studies should focus upon frail elderly participants exhibiting anabolic resistance. However, consistent with our hypothesis there was evidence of a reduced inflammatory response within the exercised muscle after MCC consumption, with decreases in NFκB protein expression after exercise in the montmorency cherry condition whereas expression of these proteins was increased in the placebo condition. The long term consequences of such changes on the responsiveness of young and older muscle to chronic training may warrant investigation, since non-steroidal anti-inflammatory medication appears to enhance resistance training induced muscle mass and strength gains in older

participants [17] whilst suppressing the acute MPS response to resistance exercise in healthy active young men [14].

Perhaps unsurprisingly, there were no changes in muscle gene expression in response to supplement, protein and/or exercise, given that Murton et al [44] found that muscle gene expression was elevated 24h after an initial bout of damaging exercise in healthy young men, but when a second identical exercise bout was performed 48h later, these changes were significantly attenuated.

In conclusion, short-term ingestion of Montmorency cherry concentrate does not significantly affect the anabolic response to protein and exercise in healthy, relatively active, older men and who are not showing signs of inflammation. However, montmorency cherry concentrate ingestion appears to reduce exercise-induced increases in muscle proteins involved in the inflammatory response.

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