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New Zealand blackcurrant extract enhances muscle oxygenation during forearm exercise in intermediate-level rock climbers

Abstract

The delivery to and utilisation of oxygenated haemoglobin to the forearm muscles are key determinants of rock-climbing performance. Anthocyanin-rich New Zealand blackcurrant (NZBC) has been suggested to improve blood flow and may enhance forearm endurance performance. As such, a double-blind, randomized, cross-over design study with 12 participants performed submaximal intermittent contractions (at 40% maximal voluntary contraction) to failure after a 7-day intake of 600 mg·day⁻¹ NZBC extract or placebo. Minimum tissue saturation index (min TSI%) was assessed during the contractions. During recovery, time to half recovery (TTHR) of TSI%, and brachial artery blood flow were assessed. There was no difference in time to exhaustion between NZBC and placebo. Min TSI% was lower with NZBC extract (43 ± 8 vs. 50 ± 11 TSI%; $p=0.007$; Cohens' $d=1.01$). During recovery, there was no effect on brachial artery blood flow. However, TTHR was faster with NZBC (26 ± 17 vs. 42 ± 26 s; $p=0.001$; Cohens' $d=1.3$) following exhaustive contractions. Seven days of NZBC extract appears to improve muscle oxygenation during and following contractions with no change in either arterial blood flow or forearm endurance performance.

Key Words: Anthocyanins; isometric exercise; ergogenic aid; vasodilation; blood flow; NIRS

Introduction

Rock-climbing performance requires forearm muscles to perform intermittent sub-maximal isometric contractions with short rest periods (Baláš et al., 2016; Fryer, Stoner, Lucero, et al., 2015). Intermittent forearm muscle contractions have a substantial aerobic energy demand, with oxidative capacity of the flexor digitorum profundus (FDP) explaining 24% of the variance in overall red-point climbing ability (Fryer et al., 2016). Furthermore, during an incremental treadwall maximum oxygen uptake test, FDP ability to offload oxygen explained 30% of the variance in red-point climbing ability (Fryer, Giles, Garrido, de la O Puerta, & España-Romero, 2017). The authors speculated that the heightened response in muscles of elite level rock climbers was caused by training adaptations allowing for greater oxygen delivery and utilisation in the forearm muscles. Thus, oxygen delivery at a macrovascular (brachial artery), and microvascular level (muscle oxygenation) are important for forearm endurance performance in climbers. One way to enhance blood flow and muscle oxygenation, and thus forearm performance, may be by intake of polyphenol ergogenic aids such as anthocyanin-rich blackcurrant (Kähkönen, Heinämäki, Ollilainen, & Heinonen, 2003).

Anthocyanins and anthocyanin-derived metabolites have been suggested to influence arterial vasodilation and relaxation (Ziberna, Lunder, Tramer, Drevenšek, & Passamonti, 2013) which may aid in decreasing peripheral resistance (Cook, Myers, Gault, & Willems, 2017) and thus increase blood flow. One-week intake of anthocyanin-rich New Zealand blackcurrant (NZBC) extract improved 16.1 km cycling (Cook, Myers, Blacker, & Willems, 2015) and repeated high-intensity running performance (Perkins, Vine, Blacker, & Willems, 2015). Blackcurrant concentrate also improved forearm blood flow in machine typists (Matsumoto et al., 2005). Only one study examined effects of NZBC extract in rock climbers (Potter et al., 2019). Potter et al. (2019) observed that 7-days loading of NZBC extract enhanced exhaustive treadwall climbing distance by 15%. It was suggested that the increase in time

to exhaustion was likely related to an increased arterial dilation causing a greater blood flow, and thus an enhanced oxygen delivery to the muscle. Therefore, we examined whether 7-day NZBC extract loading: i) enhanced forearm performance (time to exhaustion), ii) increased blood flow in the brachial artery, iii) enhanced maximal de-oxygenation in the FDP and iv) increased the capacity of the forearm to recover oxygen after exhaustive exercise (time to half recovery).

Methods

Participants

Using a double-blind, randomized cross-over design, 12 male intermediate (classified by Draper et al. (2015)) rock climbers participated. Data of two participants were removed from the forearm blood flow assessment due to technical errors with ultrasound imaging; however, they were included in other analyses. Demographic and anthropometric data is presented in Table 1. Institutional ethical approval was obtained from the University of Chichester which conformed to the Declaration of Helsinki (Puri, Suresh, Gogtay, & Thatte, 2009). Informed consent was obtained following written and verbal explanation of the procedures.

Table 1 Mean and SD demographic and anthropometric information (n = 12)

	Mean	SD
Age (years)	26	5
Height (cm)	179	5
Weight (kg)	78.3	13.4
Years Climbing	3.4	2.3
Number of Climbing Sessions Per Week	2	
Hours Training Per Week	4.88	2.58
IRCRA Grade	17.2	1.02

cm = centimetres; kg = kilogram; IRCRA = International Rock-Climbing Research Association.

Procedures

Participants were excluded if they were taking vascular acting medication or had suffered a finger injury within 6-months prior to testing. Participants were asked not to perform strenuous exercise for

24 hours, and not to consume caffeine 12 hours prior to testing. Participants used a food diary to maintain the same diet for the 7-day supplementation period. A self-selected light snack (which was kept the same for each visit) was allowed 2 hours prior to each visit. The laboratory temperature was kept constant at $\sim 20^{\circ}\text{C}$ for each visit.

Supplement protocol

Participants consumed two capsules each morning for a 7-day period with the last two capsules two hours prior to the test (Cook et al., 2017; Potter et al., 2019). Capsules were visually identical but contained either 600mg NZBC extract (i.e. 210mg anthocyanin, 35–50% delphinidin-3-*O*-rutinoside, 5–20% delphinidin-3-*O*-glucoside, 30–45% cyanidin-3-*O*-rutinoside, 3–10% cyanidin-3-*O*-glucoside) (CurraNZ[®], Health Currancy Ltd (Surrey, UK), CurraNZ Ltd (NZ)) or 600mg microcrystalline cellulose M102.

Fingerboard apparatus

The fingerboard specifications are described by MacLeod et al. (2007). The climbing hold was 55mm wide and 12mm deep, allowing participants to use an open crimp position (Schweizer and Furrer (2007)). To determine reliability of the fingerboard, 15 males not involved in the present study performed three maximal volitional contraction (MVC) trials on two separate days. The between-day coefficient of variation was 0.5% (Fryer, Stoner, Lucero, et al. (2015)). The reliability for submaximal endurance testing in rock climbers had high intra-class correlation coefficients of 0.845-0.907 (Michailov et al., 2018). In the current study, the MVC coefficient of variation for trial 1 and 2 was 4.37%.

Warm-up, familiarisation and MVC trials

Warm-up and familiarisation consisted of three phases. Phase one consisted of 5-minutes light jogging and joint mobilising. Phase two involved 5-minutes easy bouldering. Phase three involved intermittent

forearm contractions on the fingerboard with a 5kg load. During phase three, the same work-rest ratio of the trials was used (10s contraction to 3s rest). Following phase three, all participants conducted three MVC trials, each separated by 30s of active recovery. In order to allow recovery from the three phase activities and MVC trials, the NIRS equipment was fitted after the MVC's within approximately 10 minutes. In brief, participants were fitted with a continuous wave near infrared spectroscopy device (cw-NIRS), with sensors placed over the FDP. During each visit, the FDP was located using a high-resolution ultrasound device (Terason 3300, Burlington, MA, USA).

Intermittent isometric contraction protocol

After cw-NIRS setup, participants were given 5-minutes to mobilise and stretch. For the intermittent isometric contraction protocol, contractions were sustained at 40% MVC for 10s with 3s passive recovery. During the 3s rest periods, participants kept their fingers still and loosely on the climbing hold. When required, a chalk bag was offered up to the hand; it was ensured that the participant did not lower their hand to chalk, as this may affect recovery (Baláš et al., 2016). The 10:3 work-rest ratio continued until volitional fatigue, or the target force went below the 5% threshold for more than 2s. Participants received verbal encouragement throughout each trial. Once volitional fatigue occurred, participants remained still with their hand loosely resting on the hold for 3-minutes. cw-NIRS and ultrasound were used to obtain time to half recovery (TTHR) of tissue saturation index (TSI) percentage in the FDP, and total forearm blood flow (brachial artery).

Near infrared spectroscopy

A Portalite cw-NIRS device (Artinis Medical Systems BV, Elst, Netherlands) sampling at 25Hz determined the TSI% in the FDP of the dominant arm during rest (baseline), the final 26s of the 10:3s contraction/relaxation phases, and the 3-minute passive recovery. The last 26s of the contraction/relaxation phases represents the last two full contraction/relaxation cycles, which is where the lowest TSI values were previously recorded (Fryer, Stoner, Lucero, et al., 2015). Ultrasound

images showing skin, fat and muscle belly found that adipose tissue was 1.5 ± 0.97 mm deep, below the ≥ 6.4 mm that can affect NIRS signals (Van Beekvelt, Borghuis, Van Engelen, Wevers, & Colier, 2001). The cw-NIRS device consists of three light emitting diodes at 30, 35 and 40mm from a single receiver, and transmits infrared light at 760 and 850nm. TSI% was derived from the oxyhaemoglobin and de-oxyhaemoglobin, of which the sum is total haemoglobin. Due to the similarity in spectra, cw-NIRS cannot differentiate between haemoglobin and myoglobin, as such, both haemoglobin and myoglobin observations are referred to as haemoglobin.

Blood flow

Brachial artery blood velocities and diameter were measured using a Terason T3300 (Burlington, MA, USA) equipped with a 16-5MHz linear array transducer (16L5 SmartMark, Terason, Burlington, MA, USA). Standard operating procedures for blood flow measurements were followed (Stoner & Sabatier, 2012). The blood vessel was clearly extended across the entire (un-zoomed) imaging plane to minimize the risk of skewing the vessel walls. Ultrasound global (acoustic output, gain, dynamic range, gamma and rejection) and probe-dependent (zoom factor, edge enhancement, frame averaging and target frame rate) settings were standardized. The isonation angle was kept at 60° , and the sample volume included most of the vessel. Offline automated edge-detection software (Quipu, Pisa Italy) was used to identify changes in diameter and velocity waveforms over the cardiac cycle. Blood flow was calculated as the product of brachial artery cross-sectional area and the time averaged maximum blood velocity (Stoner & Sabatier, 2012). During recovery, a 30s video was recorded using LiteCam HD (New Jersey, USA) and blood flow measures started 15s after the end of the last contraction as it can take up to 15s to secure a reliable and stable video of the brachial artery following exhaustive exercise.

Sample size calculation

Sample size calculations were based upon the variable 'brachial artery blood flow' (G*Power, version 3.1). With power (1- β error probability) set at 0.8, α -level set at 0.05 and a previously reported (Cook et al., 2017) effect size of 0.82 (Cohens'*d*), a sample of 11 was required.

Data analysis

cw-NIRS data was extracted using Oxysoft (Artinis, Elst, The Netherlands). Velocity and diameter for determination of blood flow were assessed using Quipu (Pisa, Italy). As suggested by MacLeod et al. (2007) the force time integral (FTI) was used as a climbing specific measure of work done. The FTI incorporates absolute strength (MVC (N)), relative strength (40% MVC (N) = 0.4), and endurance (time to exhaustion (s)). FTI is calculated using the following formula:

$$\text{FTI} = 0.4 \times \text{force (N)} \times \text{time to exhaustion (s)}.$$

(FTI = Force Time Integral; Force (N) = Maximal Volitional Contraction; 0.4 = 40% of Maximal Volitional Contraction)

Statistical analysis was performed using Statistical Packages for Social Sciences (SPSS, Version 25). The α -level was ≤ 0.05 . Normal distribution was confirmed using the Kolmogorov-Smirnov goodness-of-fit test. Descriptive data is presented as mean and standard deviation (SD). To determine differences between the NZBC extract and the placebo trials, paired samples *t*-tests were conducted. To aid interpretation, mean difference (MD) and 95% confidence intervals (CI) were determined. Effect sizes were calculated using Cohens'*d* where 0.2, 0.5 and 0.8 represent a small, medium and large effect (Cohen, 2013).

Results

Strength and endurance characteristics

There were no significant, or meaningful differences between the NZBC extract and placebo trials for the dependent variables: MVC, MVC/body mass, time to exhaustion, and the FTI (Table 2).

Table 2 Performance data during NZBC and placebo trials (n = 12)

	NZBC		Placebo		t-test	
	Mean	SD	Mean	SD	Sig	Cohens'd
MVC (kg)	230	65	225	96	0.935	0.001
MVC/body mass (N/kg)	2.99	0.76	2.96	1.1	0.809	0.071
Time to exhaustion (s)	281	121	286	120	0.325	0.311
Force time integral	24168	6389	23629	4841	0.318	0.317

MVC = Maximal Voluntary Contraction; kg = kilogram; s = second; N = Newtons

Hemodynamic characteristics

There were no significant or meaningful differences between the NZBC extract and placebo trials for the dependent variables: baseline average TSI% (assessed during rest) and mean TSI% (assessed during contraction phases) (Table 3). Min TSI% was significantly lower in the NZBC extract trial compared to placebo (mean difference = 7, 95%CI = 2 vs. 11 TSI%). During the 3-min recovery post exhaustion, the TTHR of TSI% was significantly faster during the NZBC extract trial compared to the placebo (mean difference = 17, 95%CI = 9 vs. 25 TSI%). Lastly, there were no significant or meaningful differences between the NZBC extract and placebo trials for brachial artery blood flow, diameter, and velocity during the recovery.

Table 3 Tissue saturation index (%) assessed during baseline, the contraction phases, and the recovery period (all cw-NIRS measures are n = 12; all ultrasound forearm blood flow measures are n = 10).

Phase	NZBC		Placebo		t-test	
	Mean	SD	Mean	SD	Sig	Cohens'd
Baseline						
Average TSI (%)	83	5	81	2	0.152	0.42
Contractions						
Mean TSI (%)	54	7	59	7	0.088	0.57
Min TSI (%)	43	8	50	11	0.007*	1.01
Recovery						
TTHR (s)	26	17	42	26	0.001*	1.3
Brachial artery diameter (mm)	4.24	0.42	4.15	.39	0.464	0.22
Brachial artery velocity (m·s ⁻¹)	0.46	0.1	0.43	0.065	0.238	0.35
Brachial artery blood flow (mL·min ⁻¹)	342	117	370	130	0.298	0.34

TSI = Tissue Saturation Index; s = second; mL = millilitre; min = minute; mm = millimetre; m·s⁻¹ = meters per second. * denotes statistical significance.

Discussion

This was the first study to investigate the effects of 7-days intake of NZBC extract on hemodynamic responses to intermittent isometric forearm exercise in rock climbers. Whilst 7-day supplementation of NZBC extract improved climbing time to exhaustion during treadwall exercise (Potter et al., 2019), the physiological mechanisms remain elusive. It was suggested that this improved climbing time may have been due to an increase in forearm blood flow caused by anthocyanin-derived metabolites (Potter et al., 2019). The main findings of the present study were that although neither performance or brachial artery blood flow improved, there was a significant improvement in 1) the rate at which the FDP muscle recovers its oxygen stores (TTHR of TSI%), and 2) the ability of the FDP muscle to de-oxygenate during the final 26 s of the forearm exercise.

This is the first study to investigate arterial blood flow and muscle oxygenation responses in rock climbers with intake of NZBC extract. However, both arterial diameters (femoral artery) and de-oxygenation of Hb (vastus medialis muscle) have been studied during isometric knee extensions (Cook et al., 2017). Following a 7-day NZBC loading period, femoral artery diameter was larger, and maximal de-oxygenation lower (Cook et al., 2017). The authors suggested that NZBC extract increased dilation of the artery (diameters) which may have increased blood flow and caused a greater deoxygenation in the vastus medialis. Whilst the present study found significant improvements in muscle oxygenation during and following exercise, unlike Cook et al. (2017) there was no change in diameters, velocity or blood flow (see Table 3). This lack of change may be due to the timing of assessment. We measured blood flow for a 30s period, starting 15s after the end of exercise, as blood flow during exhaustive exercise or immediately post can be affected by turbulent flow. However, the 15s delay meant the peak blood flow response may have been missed. Finally, previous research in rock climbing observed that whilst brachial artery blood flow was greater in climbers vs. a control group (Thompson, Farrow, Hunt, Lewis, & Ferguson, 2015), it cannot distinguish between ability groups of climbers, whereas muscle oxygenation can (Fryer, Stoner, Scarrott, et al., 2015). The latter is considered to be more important to rock climbing ability than blood flow (Fryer, Stoner, Lucero, et al., 2015; Fryer, Stoner, Scarrott, et al., 2015). Therefore, because the musculature involved in isolated forearm exercise is small, the increased need to supply nutrients and remove metabolites may not be great enough to be reflected at a macrovascular level.

In line with Fryer, Stoner, Scarrott, et al. (2015), the present study suggests that changes in hemodynamic responses in rock-climbers occurs in the muscle, not the artery. Given previous research has found that TTHR explains 24% of variance in overall red-point climbing ability (Fryer et al., 2016), the faster TTHR of TSI% in the present study is an important mechanistic finding. Whilst we observed no change in arterial blood flow, dilation caused by NZBC extract in the capillary network within the FDP muscle is possible. Whilst all blood vessels are lined with endothelial cells, compared to arteries,

capillaries have much thinner walls (Moore & Ruska, 1957), which may allow for substances to more easily and quickly diffuse through them. This may explain the enhanced oxygenation seen in the forearm muscle. In addition, climbers can exhibit an enhanced capillary filtration (Thompson et al., 2015) which may also in part explain the faster TTHR.

Given that a 7-day intake of NZBC extract improved time to exhaustion over three climbs (Potter et al., 2019), our findings that neither forearm time to exhaustion, nor FTI were unchanged was unexpected. This finding was surprising given the greater level of de-oxygenation during the last 26s of the contractions. There are several potential explanations for this response. Firstly, whilst the muscle is able to de-oxygenate more with NZBC extract, the ability of intermediate climbers to recover lower oxygen stores is not sufficient to increase time to exhaustion. Fryer, Stoner, Lucero, et al. (2015) compared re-oxygenation during each 3s recovery phase in multiple ability groups of climbers and observed that intermediate climbers recovered less oxygen compared to elite and advanced climbers (Fryer, Stoner, Lucero, et al., 2015). Given that re-oxygenation assessed using NIRS has been correlated with PCr re-synthesis (McCully et al., 1994), the dose of NZBC extract in our study was maybe not sufficient to enhance intermediate climbers re-oxygenation in just 3s and thus there was no change in time to exhaustion or FTI. Future research should examine whether NZBC extract can enhance re-oxygenation and forearm performance in advanced and elite climbers. Second, forearm flexors are small in comparison to knee extensors (Cook et al., 2017) and as such, whilst the muscle may be able to off-load oxygen more during the contraction phases, it may not be sufficient to enhance ATP production from aerobic metabolism and thus there is no performance effect. Lastly, our participants were intermediate rock-climbers who may not have been able to reliably contract forearm muscles for a prolonged time. Future research should examine effects of 7-day NZBC extract in elite level climbers who have been shown to be able to regulate their contraction ability with greater accuracy (Baláš et al., 2016). In addition, research in rock climbing should focus on blood flow and oxygenation

during 1) repeated bouts of exhaustive forearm exercise, and 2) simulating rest periods during either single pitch routes and/or at belay stances during multipitch routes.

Strengths and limitations

First, we assessed intermediate rock climbers and so they may have had an inability to regulate their forearm performance; this seems probable given the coefficient of variation for MVC was 4.37%. However, as this is first study to investigate both arterial blood flow and muscle oxygen in response to rock climbing, the findings remain important. Second, whilst min TSI% was lower with NZBC extract, the mean difference was 7% and not above the SEM reported by (Baláš, Kodejška, Krupková, Hannsmann, & Fryer, 2018) of 7.6%. As such, the difference may be due to biological variability and not the supplement itself. Third, brachial artery blood flow was not assessed during the contractions and immediately after the exercise. In order to be maintain a high level of accuracy in the blood flow assessment, and due to the complexity and sensitivity of taking ultrasound measurements, it was important to have a set period of time (15s) after the cessation of exercise to ensure optimal diameter and velocity measurements.

The main findings of the present study were 1) Maximal de-oxygenation of the FDP during the last 26s (2 contractions) prior to exhaustion was lower with NZBC extract. 2) After the exhaustive forearm exercise, TTHR was faster with NZBC extract. 3) There was no change in brachial artery blood flow or performance with NZBC extract. Seven days intake is sufficient to improve forearm muscle hemodynamic responses in intermediate rock climbers.

Novelty Statement

This study is the first to measure both brachial artery blood flow and muscle oxygenation responses during isolated forearm exercise and recovery in intermediate rock climbers following 7-days NZBC extract supplementation. 7-days NZBC extract supplementation enhances muscle oxygenation

recovery post exercise, as well as during forearm exercise, with no change in brachial artery blood flow.

Practical Application Statement

Muscle re-oxygenation post exhaustive forearm exercise was faster after 7-days NZBC extract supplementation. As such, NZBC extract may be beneficial to rock climbers when a route offers rest periods during an ascent.

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The study was designed by SF, JP, CP, CG, IP and MW. Data were collected by SF, JP, CP, CG, and IP. Data were analysed by SF, JP and CP. Data interpretation and manuscript preparation was conducted by SF, JP, CP, CG, IP and MW. All authors approve the final version.