The effect of acute pomegranate extract supplementation on oxygen uptake in highly-trained cyclists during high-intensity exercise in a high altitude environment

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Abstract

Background: Recent research has indicated that pomegranate extract (POMx) may improve performance during aerobic exercise by enhancing the matching of vascular oxygen (O₂) provision to muscular requirements. POMx is rich in ellagitannin polyphenols and nitrates (NO₃⁻), which are both associated with improvements in blood flow and O₂ delivery. Primarily, this study aimed to determine whether POMx improves performance in a cycling time trial to exhaustion at 100%VO₂max (TTE100%) in highly-trained cyclists. In addition, we investigated if the O₂ cost (VO₂) of submaximal exercise was lower with POMx, and whether any changes were greater at high altitude where O₂ delivery is impaired.

Methods: Eight cyclists exercised at three submaximal intensities before completing a TTE100% at sea-level (SEA) and at 1657 m of altitude (ALT), with pre-exercise consumption of 1000 mg of POMx or a placebo (PLAC) in a randomized, double-blind, crossover design. Data were analysed using a three way (treatment x altitude x intensity) or two-way (treatment x altitude) repeated measures ANOVA with a Fisher’s LSD post-hoc analysis. Significance was set at p ≤ 0.05. The effect size of significant interactions was calculated using Cohen’s d.

Results: TTE100% performance was reduced in ALT but was not influenced by POMx (p > 0.05). Plasma NO₃⁻ were 10.3 μmol greater with POMx vs. PLAC (95% CI, 0.8, 19.7, F₁,7 = 7.83, p < 0.04). VO₂ measured at five minutes into the TTE100% was significantly increased in ALT POMx vs. ALT PLAC (+3.8 ml.min⁻¹kg⁻¹, 95% CI, −5.7, 9.5, F₁,7 = 29.2, p = 0.001, ES = 0.6) but unchanged in SEA POMx vs. SEA PLAC (p > 0.05). Submaximal VO₂ values were not affected by POMx (p ≥ 0.05).

Conclusions: The restoration of SEA VO₂ values at ALT is likely driven by the high polyphenol content of POMx, which is proposed to improve nitric oxide bioavailability. Despite an increase in VO₂, no change in exercise performance occurred and therefore this study does not support the use of POMx as an ergogenic supplement.

Keywords: Nitrates, Polyphenols, Ergogenic aids, Exercise performance

Background

Pomegranate (Punica granatum) (POM), is a seeded, red, fleshy fruit of Middle East origin which was used in traditional medicine to treat a variety of inflammatory conditions [1]. In modern-day research, the health benefits of POM have been attributed to its high concentration of nitrates (NO₃⁻) and polyphenol compounds, and consumption of POM juice (POMJ) or extract (POMx) has been linked to a decline in cancer proliferation [2], the amelioration of cardiovascular disease markers [3] and decreases in gut and joint inflammation [4, 5]. Recent research has indicated that POM-based supplements can also improve performance during aerobic exercise by enhancing the matching of vascular O₂ provision to muscular requirements [6, 7].

Polyphenols are a group of phytochemicals with antioxidant properties that contain one or more aromatic rings and at least two hydroxyl groups [8]. POMJ
contains a greater concentration of polyphenols (~3.8 mg.ml
-1) than other polyphenol-rich beverages such as red wine (~3.5 mg.ml⁻¹), Concord grape juice (~2.6 mg.ml⁻¹) and cranberry juice (~1.7 mg.ml⁻¹) [9]. These are predominantly from the ellagitannin (ET) subclass (80–90%) with smaller amounts of anthocyanins (8–15%) [10]. Consumption of ET or anthocyanin-rich foods is associated with a decrease in systolic blood pressure (SBP) and an increase in vessel diameter and blood flow [6, 11, 12] suggesting a link between POM consumption and O₂ delivery.

Supplementation with dietary NO³ influences O₂ delivery during exercise through its conversion to the potent vasodilator, nitric oxide (NO). During resting conditions, NO is primarily produced endogenously by NO synthases (NOS) [13]. However, in hypoxic conditions, such as those present locally in the muscles during exercise, activity of this pathway is limited, placing greater importance on the secondary NO³-nitrite (NO₂)-NO pathway [14]. Polyphenols further enhance the effects of dietary NO₃ by promoting their conversion into NO [15, 16], and protecting NO from damage caused by reactive oxygen species (ROS) [17].

Previous research involving NO₃ supplementation has predominantly used beetroot juice (BRI), which contains ~11 mmol.L⁻¹ NO₃ [18] in comparison to 12.93 ppm.L⁻¹ (~0.2 mmol.L⁻¹) in POMJ and 109 ppm.L⁻¹ (~1.76 mmol.L⁻¹) in POMx, as reported by Roelofs et al. [6]. Acute or short-term BRJ supplementation in low to moderately-trained individuals is associated with a large increase (>92%) in plasma NO₂, a 3–5% reduction in O₂ uptake (VO₂) during submaximal exercise and a 15–25% improvement in performance during cycling, running and knee extension time to exhaustion protocols [18–22]. In contrast, BRJ has little or no benefit on these parameters in a trained cohort, no change in performance was observed during a ten minute cycling time trial or time to exhaustion protocol in hot conditions [32]. Thus, further research is warranted to determine whether POM improves O₂ transport and endurance exercise performance.

The outcomes of the current study are primarily to further explore the effect of acute POMx supplementation on endurance exercise performance, and secondarily, to determine the effect of POMx on O₂ transport parameters. In addition, the study will investigate whether any observed effects are greater during exercise in a high altitude environment. We hypothesize that acute POMx supplementation will reduce the submaximal O₂ cost of exercise, and in doing so, improve performance in an environment (altitude) where O₂ availability may be limiting.

**Methods**

**Participants**

All participants provided written consent after being informed about the study requirements and benefits and risks of participating. A health questionnaire was also completed. Eight highly-trained cyclists, including seven males and one female, were recruited from the regional cycle community. This sample size was chosen based on previous studies which have successfully shown a decrease in VO₂ following NO₃ supplementation with a sample size of 8 participants [18, 21]. While the sample size is small, this was unavoidable given the highly-trained status of participants that we wished to study. All participants were current or past members of the national cycling or triathlon junior development programmes. Their age, height, body mass and peak aerobic capacity (VO₂max) were 17–18 years, 67.6 ± 7 kg, 180 ± 9 cm and 74.4 ± 6.2 ml.min⁻¹.kg⁻¹ respectively. The study was approved by the Massey University Human Ethics Committee (Southern A 15/54) in accordance with the Declaration of Helsinki.

**Experimental design**

**VO₂max test**

In order to determine workloads for the supplemented trials, participants completed a VO₂max test in thermo-neutral conditions (18–20 °C) at sea-level, on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), which was set up as closely as possible to the participant’s own bike. This session doubled as an initial familiarization session to ensure participants were familiar with the equipment and protocols involved in the experimental sessions. Following a five minute warm-up at 100 W, participants completed four × 7-min stages of increasing workload (e.g., 150, 200, 250, 300 W) with expired air being
collected into Douglas Bags during the last minute of each stage and analysed for O₂ and CO₂ concentrations and volume. Following a five minute active rest period, an incremental “ramp” protocol was used to determine VO₂max. Power began at 100 W and increased linearly with time (25 W.min⁻¹). Participants cycled for as long as possible and verbal encouragement was given to elicit maximal effort. As the participant’s VO₂max was approached (as indicated by a change in breathing pattern), expired air was captured in Douglas bags until exhaustion. Analysis of Douglas bags was done using a calibrated gas analysing system (AD Instruments, Dunedin, New Zealand). The gas analyser was calibrated using gases of known concentration (15.01% O₂, 5.01% CO₂). Minute ventilation (V E) and concentrations of O₂ and CO₂ values were used to calculate the volume of inspired air (V I) using the Haldane transformation, where V E was corrected for barometric pressure, ambient temperature and atmospheric water saturation. Subsequently, VO₂ and expired CO₂ (VCO₂) could be determined and are reported as standard temperature and pressure dry (STPD). The respiratory exchange ratio (RER) was calculated using VCO₂/VO₂ and attainment of VO₂max was confirmed with RER ≥1.1. A relationship between steady-state workload and VO₂ values was drawn through creation of a power curve and generation of a linear line equation y = mx + c, where m = gradient, x = power and c = start point. The equation was used to estimate power output at 50, 65 and 80% of VO₂max.

Experimental protocol

The experimental protocol (Fig. 1) was a randomized, double-blind, crossover design, which was completed on four occasions: twice in sea-level conditions (SEA) and twice in high-altitude conditions (ALT, 1657 m, ~17% O₂). The testing location was the Turoa ski-field carpark, Ohakune, which was chosen due to being the highest road accessible to our mobile laboratory in the North Island of New Zealand. Prior to each trial, participants ingested 1000 mg of either a POMx supplement (POM Wonderful LLC, USA) in capsule form or a placebo capsule of the same colour, size and shape as POMx (PLAC, brown sugar). The quantity of POMx was based on Trexler et al. [7] who showed an association between acute POMx supplementation and improved running performance. Previous analysis of the POM Wonderful LLC supplement have shown it to contain 1800 ppm polyphenols, comprised of 95.5% ellagitannins, 3.5% ellagic acid and 1% anthocyanins [32]. Participants completed a POMx trial and a PLAC trial in each environmental condition in a randomized order; the supplement blinded to the participant and the researcher. The participant was instructed to swallow the capsule whole, without tasting it, to avoid an expectation bias. In accordance with previous POMx research, the supplement was ingested 2.5 h prior to each experimental trial to allow maximal absorption prior to exercise [9]. During the 48 h prior to each session, participants were asked to limit consumption of NO₃ and polyphenol-rich foods and avoid strenuous exercise and antibacterial substances, such as mouthwash, which destroy NO₃⁻-NO₂⁻ converting bacteria on the tongue [33]. Participants consumed a standardized meal (524 cal, 8 g protein, 11 g fat, 51 g carbohydrate) three hours prior to exercise. Participants were asked to arrive at the testing session in a hydrated state, and consumed water ad libitum throughout the trial.
On arrival to the laboratory, body mass was obtained and a heart rate (HR) monitor (Garmin, Kansas, USA) was applied and recorded HR at a sampling rate of two seconds throughout the trial. After sitting in a supine position for five minutes, blood pressure of the brachial artery was measured using an automated sphygmomanometer (Japan Precision Instruments, Gumma, Japan). Blood was collected via finger prick sample (~5 μL) and analysed for blood lactate concentration ([La−]) using a blood lactate test meter (Lactate Pro, Arkray KDK, Japan). To determine haematocrit (Hct), an additional blood (~35 μL) was collected from the fingertips in heparinized capillary microtubes and immediately spun in a microhaematocrit centrifuge (Thermo IEC MB, Bellport, USA) at 14,000 rpm for two minutes. Hct was calculated as the length of red blood cells as a percentage of the total length of blood in the tube. Samples were taken in duplicate to allow calculation of an average value. In the SEA trials, a venous blood sample was also collected via venepuncture from the antecubital vein in heparinized vacutainers and immediately spun in a centrifuge (Eppendorf AG, Hamburg, Germany) at 2500 rpm for 12 min at 4 °C. Plasma was transferred into epipendorphs (Eppendorf AG, Hamburg, Germany) at 80 °C until analysis. The venous sample was not collected in ALT as samples could not be stored or analysed in the mobile laboratory.

Following the pre-exercise measures, the participant mounted the ergometer to begin cycle exercise. The experimental protocol began with three x six minute stages of stationary cycling exercise at power outputs corresponding to 50, 65 and 80% of their previously determined VO2max. In the last minute of each stage, VO2 was measured as previously described. Perceived exertion (RPE) was also recorded using Borg G [34] Scale of Perceived Exertion. Then, following a five minute rest, the load on the ergometer was increased to a workload calculated to elicit VO2max and participants were instructed to ride at this intensity, at a cadence of ≥ 80 rpm for as long as possible, with the trial being terminated once the participant could not maintain the required cadence (for the previous 10 s) or volitional exhaustion. Time to fatigue at 100%VO2max (TTE100%) was chosen in this study as a performance measure rather than a self-paced time trial to enable physiological data to be collected and compared at the five minute point. Accordingly, five minutes into the TTE100% VO2 was measured.

**Blood analysis**

Plasma samples were analysed using a Nitric Oxide Colorimetric Assay Kit (BioVision Incorporated, Milpitas, California, USA) to measure NO3 and NO2. This method is a two-step process, in which nitrate reductase is used to convert NO3 to NO2, and then Greiss Reagents convert NO2 to a deep purple azo compound. Absorbance is read at 540 nm and plotted as a function of NO3 and NO2 concentration.

**Statistical analyses**

Statistical analyses were done using statistical computer software (SPSS Statistics, Version 23, IBM Corporation, New York). Normal distribution of data was confirmed with the Shapiro-Wilk test. Variables measured during the submaximal exercise stages were analysed using three-way repeated measures ANOVA to test the significance for main effects of, and interactions between, altitude (SEA or ALT), workload (50, 65 and 80%VO2max) and treatment (PLAC or POMx). Similarly, two-way (altitude x treatment) repeated measures ANOVA was performed on all variables measured during the TTE100%. Where significant interactions (p ≤ 0.05) were observed, two-tailed paired t-tests with a Fisher’s LSD post-hoc analysis were used to identify the location of the significance. The effect sizes (ES) of significant interactions were calculated using Cohen’s d (0.1 small; 0.5 medium; 0.8 large) [35]. The relationship between VO2 and performance during the TTE100% was analysed using the Pearson correlation coefficient. Data is presented as mean ± SD or mean change and 95% CI, as appropriate.

**Results**

Participants’ self-reported adherence to supplement intake was 100% and no side effects of supplementation were recorded. There was no order effect present for any of the significant results (p > 0.05).

**TTE100% performance**

Average performances during each condition were: SEAPLAC: 10.7 ± 2 min, SEAPOMx: 12.6 ± 8.7 min, ALTPLAC: 7.0 ± 2.3 min and ALTPOMx: 8.2 ± 4.5 min. There was no main effect of treatment on performance (F1,7 = 0.776, p > 0.05). Performance was significantly decreased at ALT compared to SEA (−4.1 min, 95% CI, −6.9, −1.3, F1,7 = 12.3, p < 0.02, ES = 0.8). The individual performance responses in the TTE100% to POMx in SEA and ALT are displayed in Fig. 2. Despite no overall significant effect of POMx, there appear to be two participants (HW and LS) who increased their performance with POMx in both altitudes.

**Resting measures**

Plasma NO3 was greater following POMx compared to PLAC (+10.3 μmol, 95% CI, 0.8, 19.7, F1,7 = 7.83, p < 0.04, ES = 0.9). However, the assay used was not sensitive enough to detect changes in NO2. SBP was not significantly affected by POMx or ALT (p > 0.05).
However, there was a trend towards an increase in SBP with POMx vs. PLAC (+3.9 bpm, 95% CI, −0.6, 8.5, $F_{1,7} = 4.28$, $p = 0.08$, $ES = 0.3$). There was a strong trend towards an increase in DBP with ALT, which showed a moderate effect size (+5 mmHg, $F_{1,7} = 1.18$, $p = 0.054$, $ES = 0.6$) and a significant treatment × altitude × time interaction ($F_{1,7} = 7.64$, $p < 0.03$). However, post-hoc analyses revealed no significant differences between pre or post-exercise DBP with either treatment at either altitude ($p > 0.05$). Hct was significantly increased by ALT (+1.4%95% CI, 0.2, 2.6, $F_{1,7} = 7.56$, $p < 0.03$, $ES = 0.4$) and significantly decreased by POMx, although the effect size was small (−0.76%, 95% CI, −1.3, −0.2, $F_{1,7} = 10.4$, $p < 0.02$, $ES = 0.2$).

**Discussion**

Although acute POMx supplementation was associated with a 10.3 μmol increase in plasma NO$_3^*$ (95% CI, 0.8, 19.7, $p < 0.04$, $ES = 0.9$), its use as an acutely-ingested ergogenic supplement by highly-trained athletes is not supported by the current study, as neither performance nor submaximal VO$_2$ were significantly altered by POMx ingestion. However, our data indicated that POMx does allow maintenance of VO$_2$ at a workload prescribed to elicit 100% VO$_2$ max (at sea level) during high intensity exercise under low PO$_2$ conditions, despite no significant performance effect.

The absence of changes in performance during the TTE100% is in agreement with Trinity et al. [32] who demonstrated no effect of acute POMJ supplementation on a cycling time trial or time to exhaustion protocol in hot conditions in moderately-trained individuals. However, Trexler et al. [7] found an increase in time to exhaustion during treadmill running at 90–100% of peak

**Table 1** Variables measured during the TTE100%

| Variable            | SEA-PLAC | SEA-POMx | ALT-PLAC | ALT-POMx |
|---------------------|----------|----------|----------|----------|
| VO$_2$ (ml/min·kg$^{-1}$) | 700 ± 6.3 | 685 ± 7.2 | 632 ± 5.6$^a$ | 669 ± 5.3$^{bc}$ |
| VCO$_2$ (ml/min·kg$^{-1}$) | 724 ± 7.8 | 706 ± 8.9 | 696 ± 7.1 | 732 ± 8.0 |
| HR (bpm)            | 186 ± 8  | 175 ± 8  | 184 ± 7  | 184 ± 5  |
| [La$^-$]$_b$ (μmol·L$^{-1}$) | 11.0 ± 3.7 | 10.2 ± 2.9 | 12.2 ± 3.9 | 11.7 ± 3.9 |

Data are presented as means ± SD
VO$_2$ oxygen uptake, VCO$_2$ expired carbon dioxide, HR heart rate, [La$^-$]$_b$, blood lactate concentration, SEA-PLAC sea-level placebo, SEA-POMx sea-level pomegranate extract, ALT-PLAC high-altitude placebo, ALT-POMx high-altitude pomegranate extract

$^a$indicates $p < 0.05$ compared to SEA-PLAC
$^b$indicates $p < 0.05$ compared to SEA-POMx
$^c$indicates $p < 0.05$ compared to ALT-PLAC
velocity, in moderately trained participants, following acute supplementation of POMx (1000 mg, 30 min before exercise) at sea-level. In addition, POMx has been shown to improve performance and recovery from resistance and sprint cycling exercise [36, 37]. The current study is the first study to test POMx supplementation in highly-trained endurance athletes and is in accordance with previous research involving acute BRJ supplementation in this cohort, which found no change in performance in a running or cycling time trial in hypoxic conditions [28, 29]. However, two studies involving multi-day periods of NO$_3^-$ supplementation in low PO$_2$ conditions (11–13% O$_2$) showed changes in both O$_2$ parameters and exercise performance in moderately-trained individuals (VO$_{2\text{max}}$, 58–61 ml.min$^{-1}$kg$^{-1}$). Kelly et al. [31] found that three days of BRJ supplementation (~8.4 mmol.day$^{-1}$) decreased steady-state VO$_2$ during a bout of moderate-intensity cycle exercise by 7.6% and this resulted in an 8% improvement in a subsequent high intensity time to exhaustion protocol, compared to exercise done following the intake of a placebo. Masschelein et al. [30] found that six days of BRJ supplementation (~5 mmol.day$^{-1}$) increased arterial O$_2$ saturation by 2.7% and the muscle tissue oxygenation index in the vastus lateralis by 4% during submaximal cycling. This resulted in a 5% increase in performance during a subsequent cycling graded exercise test. Thus, the small increase in VO$_2$ with acute supplementation of POMx may not be sufficient to produce an ergogenic effect during a 100%TTE in highly-trained athletes and a longer period of supplementation may be necessary. Alternatively, the absence of change in performance despite an increase in VO$_2$ may reflect the lack of correlation between VO$_2$ and performance times ($r^2 = 0.0001$), which indicates that VO$_2$ was not the determining factor in the TTE100%. Rather, it is likely that other factors, such as anaerobic capacity, contribute more greatly to performance in a high-intensity TTE.

To the authors’ knowledge, the current research is the first study to have measured VO$_2$ during cycling exercise following supplementation with POMx. The lack of effect of POMx on submaximal VO$_2$ or VCO$_2$ values in either environment despite an increase in plasma NO$_3^-$ is in accordance with previous research involving acute supplementation with NO$_3^-$-rich BRJ in highly-trained athletes (VO$_{2\text{max}}$, $>$66 ml.min$^{-1}$kg$^{-1}$) conducted at sea-level [23, 24, 26, 38] or in low PO$_2$ conditions (13–15% O$_2$) [28, 29]. In addition, the absence of change in VO$_2$ during the TTE100% at SEA correlated with Boorsma et al. [39] who conducted a running protocol involving similar intensities to the current study, and found no significant difference in VO$_2$ values during a 1500 m TT. The restoration of an ALT-induced lowering in VO$_2$ during intense exercise following POMx compared to SEA (+3.8 L.min$^{-1}$, 95% CI, −5.7, 9.5, $p = 0.001$, ES = 0.6) differed from previous NO$_3^-$-based research involving highly-trained participants, although no other studies using hypoxia and VO$_2$ measurement have utilised a performance test protocol similar to ours. Masschelein et al. [30] found no change in VO$_{2\text{max}}$ recorded during a graded exercise test to exhaustion in hypoxic conditions (11% O$_2$, ~5000 m altitude) despite a 4% increase in the muscle tissue oxygenation index. However, in that study, exercise intensity was not stable, as it was in the current study and VO$_2$ was measured at exhaustion, rather than part-way into the exercise. In addition, MacLeod et al. [29] recorded no change in VO$_2$ during a 10 km cycling time trial completed in a normobaric hypoxic chamber (~2440 m altitude). However, the duration of this test was significantly longer than ours, which may have influenced potential effects on VO$_2$.

The changes in VO$_2$ in the current study were likely driven by the high polyphenol content of POMx, as POMx has a lower NO$_3^-$ concentration than BRJ. While BRJ-based research in highly-trained athletes has recorded increases in plasma NO$_3^-$ of 31–1907% [27, 31, 39], the current study measured a 44% increase in NO$_3^-$ with POMx. Although polyphenolic compounds are also present in BRJ, these are predominantly from the quercetin subclass, in comparison to the high ET component in POMx [40]. Previous research has generally found no link between quercetin intake, and changes in VO$_2$ or performance parameters [41, 42]. However an increase in pre or post-exercise blood flow and vessel diameter has been observed with consumption of ET or anthocyanin-containing fruit juices and extracts [6, 7, 11]. Further, Ignarro et al. [17] demonstrated that POMJ is more effective at protecting NO from breakdown than other polyphenol-containing juices and red wine, with significant antioxidant actions occurring at dilutions greater than 1000-fold. Thus, the increased VO$_2$ during the TTE100% following POMx supplementation may be due to a polyphenol-induced greater NO bioavailability.

Despite the large amount of research indicating a relationship between a supplement-induced increase in NO and changes in VO$_2$ during exercise, presumably through an increase in the efficiency of O$_2$ transport, the mechanisms behind these changes are currently irresolute. Although NO is known to mediate vasoactivity, an overall increase in vasodilation does not explain the reduction in VO$_2$ during submaximal exercise in normoxia which has been observed in other studies [18–22]. Rather, the predominant mechanistic theories to explain the effects of NO on VO$_2$ involve an increase in either the efficiency of mitochondrial O$_2$ usage or in the muscular use of ATP, affording a lower VO$_2$ requirement to sustain a given work rate [21, 43]. However, during exercise under hypoxic conditions, a NO-induced augmentation in vasodilation has
been shown to be important in maintaining blood flow to the active muscles [44]. Thus, it is likely that under hypoxic conditions, the vasoactive role of NO contributes more greatly to the level of VO2 which can be achieved during intense exercise. In accordance with previous research [26, 28], POMx did not affect [La−], indicating that any changes observed were probably not due to a change in fuel usage.

Several reasons have been proposed for the lower efficacy of NO3 supplementation on submaximal VO2 and performance in trained compared to untrained individuals. Firstly, as a response to training, athletes tend to have elevated NOS activity [45], and higher resting NO2 and NO3 values [46]. Consequently, they may have a lower requirement for NO3-NO2-NO pathway, and have sufficient NO3 present in the blood to use it when needed. Further, training adaptations which aid in O2 transport and energy production, such as increased capillarization and mitochondrial density, may reduce the incidence of acidic and hypoxic muscular environments, which decrease NOS activity, and increase reliance on the NO3 pathway [25, 47]. Finally, research in rats has suggested that the NO3 pathway is predominantly used in type II muscle fibres, which work more frequently under acidic or hypoxic conditions [48]. If this is the case, NO3 supplementation may be more effective in untrained individuals who tend to have a greater percentage of type II fibres [49]. However, currently, there is no direct evidence that type II fibres are preferentially affected in humans. Alternatively, the lack of overall performance response may be due to the presence of ‘responders’ and ‘non-responders’ to POMx. Despite no overall significant effect of POMx on performance in the current study, there were two participants who increased performance in both altitudes. Thus, a larger sample size of participants may be needed to determine the ratio of responders vs. non-responders in a highly-trained population.

In addition to the results already presented in the discussion, the current study produced two results which are difficult to explain. Firstly, in contrast to previous research which has reported a lowering in SBP following acute BRJ or ET polyphenols from grapes in sedentary or moderately-trained individuals [11, 18, 50], and no effect on SBP following BRJ supplementation in highly-trained individuals [25, 27, 29, 38], this study found a trend towards an increase in SBP with POMx (+3.9 bpm, 95% CI, −0.6, 8.5, p = 0.08). The only explanation we can give for this result is that the increase in SBP was a reactive response by the vascularature to ensure that the mean arterial pressure and thus, vessel perfusion and blood flow, were maintained, despite the NO-induced vasodilation. Further, being an acute intervention, we did not anticipate changes in Hct with POMx, but surprisingly, there was a significant decrease in Hct following POMx compared to PLAC (−0.76%, 95% CI, −1.3, −0.2), which indicated a small reduction in the O2-carrying capacity of the blood. However, despite being significant, this change had a small effect size (ES = 0.2) and considering the increase in VO2 with POMx at ALT, did not appear to affect VO2 capacity. Hct varies from day to day by ~3% [51] and it is possible that this result was due to differences in hydration between tests, as we did not measure pre-exercise hydration status, or standardize water intake during exercise. However both these explanations are purely speculative and further research is required to determine whether POMx supplementation consistently results in similar effects on SBP and Hct.

A limitation to the current study is the relatively low altitude used in the hypoxic condition compared to the altitude generally associated with physiological changes (2500–3000 m) [52] and that simulated in previous studies involving NO3 supplementation in hypoxia (~2500–5000 m) [28, 30, 31]. Due to our study being conducted in a mobile laboratory, we were restricted to areas with vehicle access in the North Island of New Zealand, and conducted the study at the highest altitude possible under these conditions. However, future studies could investigate the potential ergogenic benefit of POMx at a higher altitude of >2500 m. In addition, the current study was limited by a relatively small sample size (n = 8), which was unavoidable due to the research taking place during a high-performance junior training camp. While previous research has demonstrated a reduction in VO2 during submaximal cycling exercise [18, 21], it is acknowledged that the lack of effect of POMx on submaximal VO2 in the current study may have been due to an insufficient number of participants.

**Conclusion**

In conclusion, acute POMx supplementation allowed a partial restoration of VO2 during intense exercise in a hypoxic environment. However, no significant changes in VO2 occurred during submaximal exercise and there was no effect of POMx on performance in either environment. Thus, the results from the current study do not support POMx as an ergogenic supplement when ingested acutely prior to exercise.

**Abbreviations**

[La−]: Plasma lactate concentration; ALT: High altitude conditions; BRJ: Beetroot juice; CO2: Carbon dioxide; DBP: Diastolic blood pressure; ET: Ellagitannin polyphenols; Hct: Haematocrit; HR: Heart rate; NO: Nitric oxide; NO2: Nitrites; NO3: Nitrates; NOS: Nitric oxide synthase; O2: Oxygen; PLAC: Placebo; POM: Pomegranate; POMJ: Pomegranate juice; POMx: Pomegranate extract; RER: Respiratory exchange ratio; ROS: Reactive oxygen species; RPE: Rating of perceived exertion; SBP: Systolic blood pressure; SEA: Sea level conditions; TTE100%: Time trial to exhaustion at 100%VO2max; VO2: Volume of expired carbon dioxide; V̇E: Volume of expired air; V̇I: Volume of inspired air; VO2: Volume of oxygen consumption; VO2max: Maximal oxygen consumption
Acknowledgements

We thank the cyclists from the local cycling community who took part in the study, and the Massey University Institute of Food Science and Technology, who performed the blood analysis.

Funding

This investigation was supported by a Massey University Doctoral scholarship.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

The study was designed by EC and SS; data were collected and analysed by EC, AM and SS, data interpretation and manuscript preparation were undertaken by EC with the assistance of SS and MB. All authors read and approved the final version of the paper.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The participants were notified of all the potential risks and benefits associated with the study before written consent was obtained. The study was approved by the Massey University Human Ethics Committee (Southern A 15/54) in accordance with the Declaration of Helsinki, and written and verbal consent was obtained prior to commencement of the study.

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Received: 19 February 2017 Accepted: 26 May 2017

Published online: 31 May 2017

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