Published in final edited form as:

Appl Physiol Nutr Metab. 2014 September ; 39(9): 1038–1042. doi:10.1139/apnm-2014-0137.

The effects of pomegranate extract on blood flow and running time to exhaustion

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Abstract

Recent research has shown dietary nitrate to impart favorable effects on blood flow and exercise. The purpose of this randomized, double-blind, placebo-controlled, crossover study was to investigate the acute effects of pomegranate extract on blood flow, vessel diameter, and exercise performance in active individuals. Nineteen men and women (Mean ± SD; Age: 22.2 ± 2.2 yrs; Height: 174.8 ± 10.7 cm; Body mass: 71.9±13.5 kg) were randomly assigned to a placebo (PL) or pomegranate extract (PE) group. Participants performed a maximal oxygen consumption treadmill test to determine peak velocity (PV). Participants returned after 24–48 hours, and ingested either PL or PE. Brachial artery blood flow was assessed using ultrasound at baseline and 30 minutes post-ingestion (30minPI). Three treadmill runs to exhaustion (TTE) were performed at 90%, 100%, and 110%PV. Blood flow was assessed immediately after each exercise bout and 30 minutes post-exercise (30minPEx). After a 7–10 day washout, participants repeated the same procedures, ingesting the opposite supplement. Separate repeated measures ANOVAs were performed for blood flow, vessel diameter, and TTE. Blood flow was significantly augmented ($p=0.033$) 30minPI with PE in comparison to PL. Vessel diameter was significantly larger ($p=0.036$) 30minPEx with PE. Ingestion of the PE was found to significantly augment TTE at 90% ($p=0.009$) and 100% PV ($p=0.027$). Acute ingestion of PE 30 min prior to exercise may enhance vessel diameter, blood flow, and delay fatigue during exercise. Results of the current study indicate that PE is ergogenic for intermittent running, eliciting beneficial effects on blood flow.

Keywords

nitrate; nitrite; nitric oxide; polyphenol; vasodilation; exercise hyperemia; vessel diameter; flow-mediated dilation; mitochondrial efficiency; exercise tolerance

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Introduction

During exercise, the demand for oxygen and energy substrates is elevated in active skeletal muscle. To meet increased demand, blood flow to working musculature is increased in a process known as exercise hyperemia (Hellsten et al. 2012). Exercise hyperemia is accomplished by a number of central and peripheral cardiovascular adjustments affecting blood flow; nitric oxide (NO) has been identified as an important contributor to the vasodilation observed with exercise (Hellsten et al. 2012).

The nitric oxide synthase (NOS)-dependent pathway of NO production includes a series of reactions oxidizing L-arginine to L-citrulline and NO (Bailey et al. 2011). Reactions in this pathway are catalyzed by NOS enzymes, and require the presence of a number of substrates and cofactors including oxygen and L-arginine (Bailey et al. 2011). Given their importance in the NOS-dependent pathway, a number of studies have investigated the effects of arginine and citrulline-based supplements on exercise performance (Bescos et al. 2012; Camic et al. 2010; Hickner et al. 2006). An alternative, NOS-independent pathway of NO production has been identified, in which nitrate (NO_3) is reduced to nitrite (NO_2), and further reduced to NO (Bailey et al. 2011). The NOS-independent pathway can function anaerobically and is stimulated by hypoxia and hydrogen ions, potentially indicating a significant role in NO formation during exercise (Bailey et al. 2011). Previous studies on nitrate supplementation have reported reduced blood pressure (Siervo et al. 2013), oxygen uptake (Bailey et al. 2011), and blood lactate (Ferguson et al. 2013); enhanced exercise tolerance (Bailey et al. 2009), exercise performance (Bescos et al. 2012), and mitochondrial efficiency (Larsen et al. 2011); and increased oxygenation and blood flow to skeletal muscle (Ferguson et al. 2013). Several exogenous food sources have been purported to naturally augment this pathway. Beetroot juice has recently been shown to be an ergogenic source of supplemental nitrate, and pomegranate extract (PE) is another viable source, with very high nitrate and polyphenol concentrations and research supporting similar cardiovascular effects (Aviram and Dornfeld 2001; Aviram et al. 2004; Sumner et al. 2005). Aside from their dietary nitrate content, the effects observed in previous pomegranate and beetroot research may also be attributed, in part, to polyphenol content. Supplementation with polyphenols has been shown to increase blood flow and vessel dilation (Barona et al. 2012). These results indicate potential for an acute ergogenic effect, and may be explained by enhanced endothelial NOS expression and NO production (Leikert et al. 2002).

The purpose of the current study was to investigate the effects of acute PE ingestion on vessel diameter, blood flow, and exercise performance. Previous studies have required participants to consume a nitrate-rich supplement chronically or multiple hours before exercise (Bailey et al. 2011; Bescos et al. 2012), but the high nitrate and polyphenol concentration of PE may allow for a more convenient timing protocol. The current study aimed to investigate the effects of acute PE ingestion 30 minutes prior to an exercise bout, utilizing highly active participants. It was hypothesized that ingestion of the PE supplement would enhance vessel diameter and blood flow before and after exercise. It was also hypothesized that PE ingestion would enhance submaximal aerobic exercise performance.

Materials and methods

Subjects

Nineteen (10 male, 9 female) highly active participants (Mean ± SD; Age: 22.2 ± 2.2 yrs; Height: 174.8 ± 10.7 cm; Body mass: 71.9±13.5 kg; VO_2max: 51.3 ± 9.4 ml·kg^{-1}·min^{-1}) volunteered to participate in this study. All participants participated in a regular exercise program for at least two months prior to the study, and were not consuming any prescription drugs or supplements that could potentially confound the results of the current study. Exercise status was confirmed with average baseline VO_2max values of 44.3 ± 3.2 ml·kg^{-1}·min^{-1} for women and 57.7 ± 8.6 ml·kg^{-1}·min^{-1} for men, which according to ACSM's guidelines (Pescatello and American College of Sports Medicine 2014) classifies the participants in the top 80th and 90th percentile, respectively, for aerobic capacity. All ethical guidelines and principles set forth by the Declaration of Helsinki and the Office of Human Research Ethics were followed. All methodology was approved by the University's Institutional Review Board, and all participants signed an informed consent prior to participation.

Experimental design

This study used a randomized, double-blind, placebo-controlled, crossover design. Participants were asked to abstain from exercise and any pre-workout supplement for 24 hours, as well as caffeine, mouthwash, and food for 3 hours prior to all exercise testing. Three-day diet logs were completed and analyzed using The Food Processor software (ESHA Research, Salem, OR, USA). Participants were asked to replicate their recorded food log intake to ensure similar dietary intake between trials. Each participant completed a baseline maximal graded exercise test to exhaustion to determine maximal oxygen consumption (VO_2max) and peak velocity (PV). Two sets of three treadmill runs to exhaustion were completed to determine critical velocity (CV) and anaerobic running capacity (ARC). A minimum of seven days separated each set of runs, allowing for a washout phase. Blood flow was measured using a GE Logiq-e B-mode ultrasound (GE Healthcare, Wisconsin, USA) after each run to exhaustion. A visual analog (pain) scale (Campbell and Lewis 1990) was completed 30 minutes post-ingestion of PE or PL and immediately post CV test; participants were also asked to complete a vitality scale (Bostic et al. 2000) 30 minutes post-ingestion of PE or PL.

Maximal oxygen consumption (VO_2max)

To determine VO_2max and PV, all participants performed a graded exercise test to volitional exhaustion on a treadmill (T2100, GE Healthcare, Wisconsin, USA). As previously described (Peake et al. 2004; Smith et al. 2010), to obtain VO_2max, the treadmill was set at 10 km/h for the initial stage and increased by 2 km/h every two minutes until 16 km/h. At 16 km/h the stages increased 1 km/h every minute until 18 km/h, upon which percent grade was increased every minute until volitional exhaustion. The test was considered maximal if it met a minimum of two of the following criteria: a plateau in heart rate (HR) or HR within 10% of maximal HR; a plateau in VO_2 or an increase of no more than 150 ml·min^{-1}; a respiratory exchange ratio value greater than 1.15 (Pescatello and American College of Sports Medicine 2014). Maximal oxygen consumption was determined by indirect

calorimetry with a metabolic cart (ParvoMedics, Sandy, UT) using a mouthpiece and tube connection to analyze the breath-by-breath expired gases, in 15-sec averages. The highest recorded value was recorded as VO_2max.

Supplementation

Participants ingested either 1000 mg of pomegranate extract (PE; TRUE Pomegranate Extract (NITRO$_2$GRANIT™), Stiebs Nature Elevated, Madera, CA) or placebo (PL; 95% maltodextrin, 5% purple carrot and hibiscus for color). Both PE and PL were given in 2, 500mg capsules with 6 ounces of water. Following ingestion, participants were asked to wait 30 minutes prior to beginning the CV test to allow sufficient time for circulating nitrate and nitrite to become elevated. Treatment order was randomized with half of the participants ingesting the active treatment during their first session, and the rest in the latter session.

Critical velocity

Each participant performed three treadmill runs to exhaustion. Runs were completed at 90%PV, 100%PV and 110%PV of the treadmill velocity that corresponded to their peak speed determined by the VO_2max test. Intensities were randomly ordered for each participant, but maintained between both trials. A rest period of 15 minutes was allotted between each run in order for HR to return within 10% of resting HR. The time to exhaustion was recorded for each run. To determine CV and ARC, the linear total distance model was used, previously described by Florence and Weir et al. (Florence and Weir 1997) and Smith et al. (Smith et al. 2010):

$$\mathrm{TD}=\mathrm{ARC}+\mathrm{CV}\cdot\mathrm{t}$$

where total distance (TD) during each run was plotted over time to exhaustion (TTE). A linear regression was then used to calculate the y-intercept (ARC), and the slope of the line of best fit (CV). Calculations were performed in custom-written Labview software (National Instruments, Version 9.0, Austin, TX, USA).

Brachial artery blood flow

Blood flow and vessel diameter were performed during both CV tests. Immediately upon arrival participants were asked to be seated; a blood pressure cuff (700-11ABK, ADC, New York, USA) was placed around the upper right arm and inflated to 180 mm Hg for 35–40 seconds (Birk et al. 2013). Once the cuff was removed, the participant's arm was positioned approximately 80° from the torso, and a wide-band linear array ultrasound transducer probe (GE: 12L-RS; 5–13 mhz) was held stable against the skin with sufficient pressure to obtain a clear image of the brachial artery without compressing the dermal layer. The ultrasound was set to view continuous blood volume flow in the vascular, pulse wave, and colorflow setting. Once the brachial artery was found, a minimum of four pulses occurred before freezing the ultrasound image. Diameter was then measured using the ultrasound software by a straight-line measure of distance between the walls of artery. Once diameter of the vessel was measured, blood volume flow was estimated using the ultrasound software. The procedure for measurement of blood flow and vessel diameter was taken at baseline (base), and 30

minutes following ingestion (30minPI) of PE or PL. Additional measurements were taken immediately following each of the three runs to exhaustion (IP90%, IP100% 1P110%), and 30 minutes post-exercise (30minPEx); the blood pressure cuff was not used following the exercise bouts or 30minPEx. Re-test reliability for blow flow and vessel diameter determined from this lab have demonstrated ICC values of 0.66 and 0.83 and SEM values of 8.57 $ml \cdot min^{-1}$ and 0.028 cm, respectively.

Statistical analyses

Two-way repeated measures ANOVAs (time × treatment) were used to evaluate TTE, and VAS and vitality scale questions. Separate repeated measures analyses of variance [time (base vs. 30minPI vs. IP90% vs. IP100% vs. IP110% vs. 30minPEx) × treatment (PE vs. PL)] were performed for vessel diameter and blood flow. Analyses were performed using SPSS (Version 20.0; IBM, Somers, NY, USA).

Results

Table 1 presents change in time to exhaustion (sec) after ingestion of the PE and PL. Ingestion of the PE was found to significantly augment TTE at 90% (387.9 ± 199.2 vs. 346 ± 162.5 sec, p = 0.009) and 100% PV (170.8 ± 66.3 vs. 159.3 ± 62.3, p = 0.027) (Table 1). However, PE did not produce a significant effect on CV (p = 0.096) or ARC (p = 0.106). The visual analog (pain) scale was not significantly different between treatments. However, on the vitality scale, the following statement, “At this moment I feel alive and vital” was found to be significantly greater (p = 0.037) 30 min following ingestion PE compared to PL.

Vessel diameter was significantly increased (p = 0.036) 30min post-exercise with the consumption of PE (VD = 0.42 ± 0.07 cm) in comparison to PL (VD = 0.39 ± 0.07 cm) (Table 2). Blood flow was significantly augmented (p = 0.033) 30min post-ingestion of PE (BF =40.6 ± 24.8 ml/min) in comparison to PL (BF = 29.6 ± 24.9 ml/min) (Table 3). No other time points were significantly different as a result of PE ingestion.

Discussion

Compared to PL, acute ingestion of PE resulted in a significant increase in TTE at 90% and 100% PV. Vessel diameter 30minPEx and blood flow 30minPI were significantly augmented following ingestion of PE. Participants also reported significantly greater feelings of vitality after PE compared to PL, which may have important implications for exercise tolerance. Previous research has indicated that supplementation with dietary nitrate (Ferguson et al. 2013) and polyphenols (Barona et al. 2012) may increase blood flow and vessel dilation, which could contribute to the ergogenic effect of PE.

Improvements in TTE at 90% (Δ = 41.9 ± 58.4; p = 0.009) and 100% (Δ = 11.6 ± 21.0; p = 0.027) of PV suggest an ergogenic effect from PE. This finding is consistent with previous studies reporting improvements in high-intensity exercise as a result of nitrate supplementation (Bailey et al. 2011; Bescos et al. 2012), and supports the hypothesis that acute ingestion of 1000 mg PE would be ergogenic in trained participants. Supplementation with dietary nitrate has been shown to enhance exercise efficiency by decreasing oxygen

consumption during exercise without limiting performance (Bailey et al. 2011), increasing blood lactate (Ferguson et al. 2013), or increasing energy contribution from glycolysis or the phosphagen system (Bailey et al. 2010). This enhanced efficiency may contribute to the increased TTE observed in the present study. It has been previously demonstrated that NO plays a role in modulating mitochondrial respiration (Castello et al. 2006), and that inhibition of endogenous NO production increases oxygen consumption at rest (Shen et al. 1994). Bailey et al. (Bailey et al. 2010) showed that nitrate supplementation prior to exercise decreased total ATP turnover, indicating that the reduced oxygen cost of exercise may be attributed to a reduction in the ATP cost of force production. Larsen et al. (Larsen et al. 2011) concluded that increased mitochondrial efficiency may reduce oxygen cost of exercise following nitrate supplementation. Despite improvements in TTE at 90% and 100% of PV, no improvement was observed at 110%. Given the potential ergogenic mechanisms of pomegranate extract involving oxygen delivery and mitochondrial efficiency, it is possible that PE supplementation preferentially enhances aerobic exercise capacity compared to higher-intensity anaerobic activities; more research is needed to confirm this observation.

Many studies have investigated the effects of various NO-related supplements on exercise performance, targeting both pathways of NO production. L-arginine is the direct precursor to NO in the NOS-dependent pathway, prompting researchers to investigate a potential ergogenic effect of L-arginine-based supplements. Although some evidence has indicated an ergogenic benefit from L-arginine supplementation, this effect appears to be training status-dependent. While positive findings have been reported in untrained and moderately-trained participants, an ergogenic effect is not likely in well-trained athletes (Bescos et al. 2012).

Although L-arginine is the direct NOS-dependent precursor to NO, L-citrulline supplementation increases plasma L-arginine concentrations more effectively than L-arginine supplementation (Schwedhelm et al. 2008). This has led to speculation that L-citrulline-based supplements may improve performance more effectively than L-arginine. Although Perez-Guisado and Jakeman (Perez-Guisado and Jakeman 2010) showed that an acute dose of citrulline malate increased work capacity in moderately-trained participants completing a resistance training protocol, this effect cannot be conclusively attributed to NO-related mechanisms. Conversely, an acute dose of L-citrulline was shown to have an ergolytic effect by decreasing TTE on an incremental treadmill test in moderately-trained participants (Hickner et al. 2006). More research is needed to evaluate the effects of L-citrulline supplementation on blood flow and exercise performance.

Initial research in nitrate-rich ingredients like beetroot and pomegranate has demonstrated ergogenic effects. Beetroot has been shown to improve performance and reduce the oxygen cost of exercise (Bailey et al. 2011; Bescos et al. 2012). Further, the ergogenic effect of beetroot does not appear to apply exclusively to untrained populations; Lansley et al. (Lansley et al. 2011) reported increased power output and enhanced time trial performance following a single acute dose in competitive cyclists. While previous studies with pomegranate juice have documented improved recovery and decreased soreness following eccentric exercise in recreationally active (Trombold et al. 2010) and resistance-trained (Trombold et al. 2011) males, the current study supports the hypothesis of an ergogenic effect from PE, and features a convenient protocol of acute ingestion 30 minutes prior to an

exercise bout. Past research with beetroot has required participants to follow somewhat inconvenient dosing protocols, consuming the product chronically, or 2.5 hours before exercise (Bailey et al. 2011; Bescos et al. 2012). Pomegranate extract is a highly concentrated source of dietary nitrate and polyphenols, which may allow for a significantly shorter time between supplement ingestion and an observable ergogenic effect. The current study also utilized a high-intensity, intermittent exercise protocol that may simulate a number of sports more effectively than a single time trial.

Beetroot juice supplementation has been shown to increase limb blood flow in exercising rats (Ferguson et al. 2013). Our results support previous research demonstrating enhanced vasodilation and blood flow in response to nitrate and polyphenols. Thirty minutes after exercise, vessel diameter was significantly greater with PE consumption in comparison to PL (0.42 ± 0.07 cm vs. 0.39 ± 0.07 cm; $p = 0.036$). Further, blood flow measured 30 minutes post-ingestion was significantly greater in the PE condition compared to PL (40.6 ± 24.8 ml/min vs. 29.6 ± 24.9 ml/min; $p = 0.033$). The vasodilatory effect of PE supplementation may contribute to the ergogenic effect observed in the present study. Enhanced vasodilation is likely to improve metabolic control and oxidative function by increasing oxygen delivery relative to consumption, increasing the oxygen driving pressure from the capillary to the myocyte (Ferguson et al. 2013). While increases in vessel diameter and blood flow were not found uniformly at every time point, it is possible that the hyperemic response to exercise concealed any significant changes to blood flow or vessel diameter in the measurements taken immediately after each run to exhaustion. Conversely, significant effects on flow and vessel diameter were observed at rest, both 30 minutes after PE ingestion and 30 minutes after exercise. While research in beetroot juice has shown enhanced blood flow to exercising skeletal muscle (Ferguson et al. 2013), the high concentration of nitrate and polyphenol content in PE may further augment the delivery of blood, oxygen, and energy substrates to active musculature.

The effect of PE on vasodilation and blood flow may also have significant implications for recovery from exercise. Vessel diameter in the PE condition was significantly greater than PL 30 minutes post-exercise, which could aid in exercise recovery by enhancing nutrient delivery to skeletal muscle. This may be a plausible mechanism for the improved recovery observed in previous studies utilizing pomegranate juice (Trombold et al. 2010; Trombold et al. 2011). Aside from the well-documented effects of nitrate on blood flow, this effect may also be attributed to the polyphenol content of PE. Evidence suggests that polyphenols increase flow-mediated vasodilation (Barona et al. 2012), likely via increased endothelial NOS expression and NO production following use of polyphenols (Leikert et al. 2002). Previous studies have suggested that polyphenol-rich ingredients may enhance recovery from damaging exercise (Malaguti et al. 2013). While research with pomegranate juice has indicated improved recovery from exercise (Trombold et al. 2010; Trombold et al. 2011), more research is needed to investigate the effects of PE on exercise recovery and the underlying mechanisms. In the present study, participants in the PE condition also reported higher scores on a subjective vitality scale ($p = 0.037$), suggesting a potential value to individuals engaged in rigorous training programs or clinical populations with a low tolerance for exercise.

Results of the present study indicate that PE enhances vessel diameter and blood flow with a concomitant increase in TTE, thus implicating PE as a promising ergogenic aid in the context of high-intensity intermittent running. Results of the current study indicate that further research on PE is warranted. Research regarding optimal dosing and timing strategies may enhance the effects of PE on blood flow and performance. Future research may also help to determine if the ergogenic effect of PE supplementation is applicable to other exercise modalities and intensities, and if chronic PE supplementation confers more pronounced effects. Previous research has shown pomegranate juice to decrease blood pressure (Aviram and Dornfeld 2001; Aviram et al. 2004) and increase myocardial perfusion (Sumner et al. 2005), while beetroot has been shown to improve exercise tolerance in peripheral artery disease (Kenjale et al. 2011). More research is needed to determine if the results of the present study translate to a significant improvement in exercise tolerance, blood pressure, and tissue perfusion in clinical populations.

In conclusion, ingestion of PE in close proximity to an exercise bout led to enhanced vessel diameter, blood flow, and delayed fatigue in highly active participants. These effects are likely attributed to PE's high nitrate and polyphenol content, which have been shown to increase nitric oxide production and enhance exercise efficiency. Whereas previous research has required participants to ingest sources of dietary nitrate chronically or multiple hours before exercise, PE ingestion resulted in an ergogenic effect when ingested 30 minutes prior to the bout. Results of the current study indicate that PE is an efficacious ergogenic aid for sports involving intermittent running, eliciting beneficial effects on blood flow and exercise performance as few as 30 minutes after ingestion. More research is needed to evaluate the effects of chronic PE supplementation and potential clinical applications.

Acknowledgments

This study was funded by Dymatize Enterprises LLC, Bedford, TX, USA and Stiebs Nature Elevated, Madera, CA, USA. The project described was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant *1KL2TR001109*. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Table 1

Mean ± SD of the change in time to exhaustion (TTE) for the three treadmill runs

%	PL TTE (sec)	PE TTE (sec)	ΔTTE (sec)	P-value
90	346 ± 162.5	387.9 ± 199.2	41.9 ± 58.4	0.009[a]
100	159.3 ± 62.3	170.8 ± 66.3	11.6 ± 21.0	0.027[a]
110	104.4 ± 40.1	108.8 ± 45.1	−4.4 ± 15.8	0.244

[a]Significant change in TTE from PE to PL

Table 2

Mean ± SD of the change (PE-PL) in vessel diameter for ultrasound measures

	PL Diameter (cm)	PE Diameter (cm)	Diameter (cm)	P-value
30minPI	0.38 ± 0.07	0.40 ± 0.07	0.02 ± 0.07	0.217
30minPEx	0.39 ± 0.07	0.42 ± 0.07	0.021 ± 0.04	0.036[a]
IP90%	0.41 ± 0.08	0.41 ± 0.05	−0.003 ± 0.05	−0.828
IP100%	0.40 ± 0.07	0.42 ± 0.07	0.02 ± 0.04	0.054
IP110%	0.40 ± 0.08	0.40 ± 0.07	0.004 ± 0.06	0.783

[a] Significantly different from PE to PL

Table 3

Mean ± SD of the change in blood flow (BF) for ultrasound measures (PE-PL)

	PL BF (ml/min)	PE BF (ml/min)	ΔBF (ml/min)	P-value
30minPI	29.6 ± 24.9	40.6 ± 24.8	11.0 ± 20.8	0.033[a]
30minPEx	47.7 ± 23.3	58.5 ± 50.8	10.7 ± 44.8	0.311
IP90%	109.8 ± 71.4	103.5 ± 60.9	−6.3 ± 43.5	0.537
IP100%	85.8 ± 40.4	116.2 ± 77.0	30.3 ± 78.3	0.109
IP110%	96.8 ± 45.4	99.1 ± 32.3	2.3 ± 51.2	0.847

[a]Significant change in blood flow from PE to PL