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Consumption of Pomegranate Juice Attenuates Exercise - Induced Oxidative Stress, Blood Pressure and Urinary Cortisol/Cortisone Ratio in Human Adults

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Abstract

Background: Oxidative stress is exacerbated in overweight and obese individuals after acute exercise compared with their non-obese counterparts. Antioxidants supplementation of the diet may be one intervention to reduce exercise-induced oxidative stress in this vulnerable population. The aim of this study was to investigate whether polyphenol-rich pomegranate juice attenuates post-exercise oxidative stress and contributors to oxidative stress (glucocorticoids) and blood pressure in healthy overweight subjects.

Methods: Males and females participated in a randomized placebo controlled parallel pilot-study (mean BMI: 26.7 ± 6.6 kg/m^2). Two groups of (n = 12) participants received either pomegranate pure juice (500 mL/day containing total polyphenols of 1685 mg GAE/L) or placebo (water matched for total energy) and all participants completed two standardized 30 min treadmill tests (50% W_{max}) at baseline and after one week of the intervention.

Results: Exercise induced lipid peroxidation (MDA) was lower following pomegranate juice consumption compared with placebo (31.2 ± 10.6 to 26.5 ± 9.8 MDA µmole/day) after 1 week (P = 0.035). Urinary free cortisol was reduced from 179.4 ± 53.2 to 125.6 ± 43.5 nmole/24h which was significant (p = 0.042). In addition, there was a statistically significant increase in urinary free cortisone: from 112.2 ± 40.4 to 187.6 ± 90.2 nmole/24 h (p = 0.045), and a significant decrease in the urinary free cortisol/cortisone ratio (p=0.009) from 1.6 ± 1.1 to 0.67 ± 0.55 following one week of pomegranate juice intake. Pomegranate juice consumption was also found to decrease systolic blood pressure pre-exercise (136.7 ± 11.7 to 131.8 ± 8.8 mmHg (p = 0.007), and post-exercise from 158.8 ± 15.8 to 148.1 ± 12.3 mmHg (p < 0.01) and diastolic blood pressure (86.3 ± 10.6 to 82.5 ± 6.8 mmHg (p = 0.04) and 103.1 ±12.5 to 93.9 ± 11.5 mmHg (p = 0.001), pre and post exercise, respectively. Correlation results between the change in Cortisol/cortisone ratio with the effect on blood pressure showed a negative significant association post pomegranate juice intake (p = 0.028 for systolic and p = 0,008 for diastolic BP). There were no changes in lipid peroxidation or blood pressure following placebo treatment.

Conclusions: These findings suggest that pomegranate juice consumption prior to an acute bout of moderate intensity exercise can alleviate Blood pressure and exercise-induced oxidative stress in the overweight and obese population.

Keywords: *Pomegranate juice; Blood Pressure; Glucocorticoids; Exercise; Oxidative Stress*

Abbreviations

AOX: Antioxidant; BP: Blood Pressure; CVD: Cardiovascular disease; DBP: Diastolic Blood Pressure; ELISA: Enzyme-Linked Immune Sorbent Assay; GC: Glucocorticoids LDL: Low Density Lipoprotein; MDA: Malonaldehyde PJ: Pomegranate (pomegranate pure) Juice ROS: Reactive Oxygen Species SBP: Systolic Blood Pressure TBARS: Thio barbituric Reactive Substances

Introduction

Oxidative stress is defined as an imbalance between the antioxidant and pro-oxidant processes that occur during metabolism resulting in an increase in the production of free radicals and reactive oxygen species (ROS) [1-3]. An accumulation of oxidative by-products over time may lead to oxidative damage of biological molecules including lipids, proteins and DNA [4]. It is well known that acute exercise particularly of a moderate to high intensity promotes oxidative stress which is exacerbated in overweight and obese individuals [5,6]. The prevalence of overweight is increasing globally and has become a serious public health concern. Overweight increases the risk of chronic metabolic diseases including hypertension, cardiovascular disease and type 2 diabetes [7-9]. Low-grade inflammatory status in overweight individuals has been proposed as one of the mediating processes in the development of metabolic disease and several studies have reported an association between oxidative stress and inflammation in these conditions [10-12]. It is thought that a compromised endogenous antioxidant defence system in this population enhances free radical production and may contribute to further oxidative damage during exercise [13-14]. Studies have found that blood levels of specific antioxidants including vitamins C and E, and total antioxidant status are lower in obese than non-obese individuals [15-18]. Thus, it was concluded that dietary factors represent a key component of the disease process and specific dietary constituents are considered to be important to health.

Antioxidant supplementation may therefore minimise exercise-induced oxidative stress in the vulnerable overweight and obese populations. However, evidence for this has shown conflicting results especially following administration of single antioxidant supplements which in some cases have been shown to promote pro-oxidation processes [19-22]. As a consequence, the emphasis placed on the benefits of antioxidants derived from food products has been important. Dietary polyphenols have received much attention in this regard as they have the ability to neutralize free radicals and ROS. Consumption of Green tea has been shown to effectively inhibit LDL oxidation and increase serum antioxidant activity [23,24].

Furthermore, polyphenol-rich dark chocolate alleviated blood pressure and its complications in overweight and obese individuals [25,26]. Pomegranate (Punica granatum L) provides a rich and varied source of polyphenols. Several studies have demonstrated that consumption of pomegranate juice can exert positive effects on cardiovascular risk; hypertension, atherosclerosis, endothelial dysfunction and inflammation [27-33]. In addition, some researchers have shown that PJ consumption reduces carotid intima-media thickness and blood pressure [34]. As powerful antioxidants, phenolics may protect the body from damaging oxidation reactions [37-39] however other mechanisms may also operate which are currently under investigation. In a limited study of hypertensive patients, consumption of pomegranate juice for two weeks was shown to reduce systolic blood pressure by inhibiting serum angiotensin-converting enzyme [38]. Obesity and diabetes are other underlying factors linked to metabolic and cardiovascular risk [39,40], as well as abnormal cortisol levels and metabolism [41-43].

The importance of cortisol in regulating blood pressure has been highlighted in several conditions, and Chronic excessive activation of the glucocorticoid receptor (GR) is known to induce obesity, insulin resistance, glucose intolerance and hypertension [42,44,45] In Glucocorticoids exert a direct effect on the heart and blood vessels via 11β HSD enzymes type 1 and 2. Increased 11β HSD-1 activity is implicated in the development of the metabolic syndrome and identifying dietary constituents that influence 11β HSD-1 activity could lead to novel methods of preventing CVD and associated risk factors. The association between excess cortisol and various parameters of the metabolic syndrome, including hypertension and insulin resistance, is now increasingly recognised [45,46]. The purpose of this study was to determine whether consumption of polyphenol-rich pomegranate juice lowers oxidative stress in healthy overweight adults fol-

lowing a single acute bout of moderate intensity exercise. A secondary purpose was to determine whether blood pressure and urinary glucocorticoids are attenuated by pomegranate juice supplementation in this population.

Methods and protocols

Participants

Free-living male and female volunteers (n = 24) who were apparently healthy participated in this study. Subjects were recruited from the University following an advertisement through the internal moderator email system. All participants had to meet the following criteria before enrolment in the study: no participation in regular physical activity (vigorous exercise two times or more per week); no chronic health problems or current smoking; no history of cardiovascular, metabolic, or respiratory disease and no consumption of antioxidant supplements within the past 6 months. Written informed consent was provided by each participant consistent with the university policy on the protection of human subjects. The protocol of the study was approved by the Research Ethics Committee at Queen Margaret University (UK).

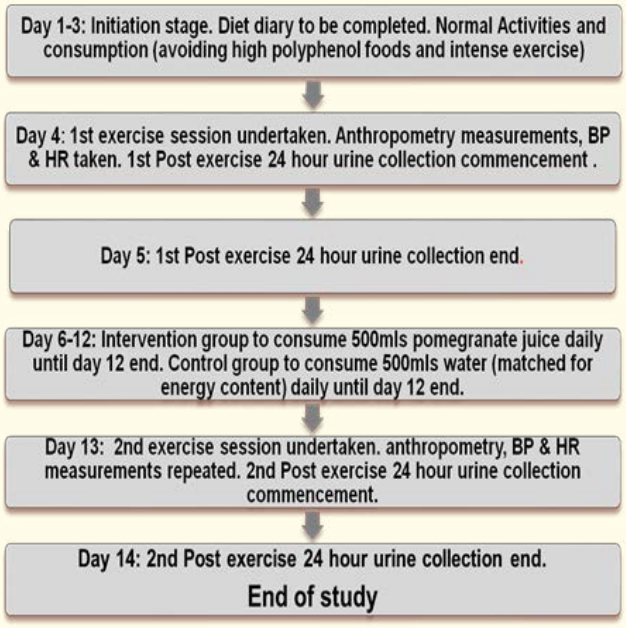

Figure 1: *Outline of the study protocol.*

Study design

The study was a randomised, single-blind, placebo-controlled parallel group trial. All participants visited the clinical facility on two occasions where they performed a 30 minutes moderate treadmill exercise session (50% W_{max}) pre and post consumption of pure PJ (500 mL daily containing 1685 mg total phenolics L^{-1}) or placebo. During each visit anthropometric measurements were taken. Height and weight were measured using a standard medical grade scale. Waist circumference was measured using a metal measuring tape at anatomical landmarks described by the American College of Sports Medicine [47]. BMI values were determined as overweight and obese (BMI ≥ 25 kg/m^2) and participants were randomised into two groups: placebo and PJ. Groups assigned to receive PJ were provided with a 500 ml bottle per day (containing 1685 mg total phenolics/L). The placebo (water) was matched for volume and energy. All liquid samples

were provided to participants in dark coloured opaque bottles. The dose of polyphenols in the PJ was chosen based on previous studies that showed reductions in SBP and DBP and urinary glucocorticoids, in healthy adults [33]. Treatment was administered for a period of one week. All 24 participants completed this study.

Measurements

Glucocorticoids

Twenty-four-hour urine samples were collected from volunteers into sterile plastic containers after each exercise session at baseline and after 1 week of the juice or placebo intake. The weight of each collected sample of urine was measured and a 20 mL aliquot was taken and stored at -80°C until analysis.

Cortisol and cortisone levels in urine samples were estimated by using highly specific and sensitive ELISA methods previously published [48,49]. All samples from the same subject were tested on the same day to prevent any inter-day variation. The data were then used to calculate cortisol-to-cortisone ratio which serves as an index of 11β-Hydroxy steroid dehydrogenase activity. Monitoring the activity of this enzyme helps detect changes in peripheral metabolism of cortisol [46].

Thio barbituric acid reactive substances (TBARS)

The TBARS assay was performed for the assessment of oxidative stress in urine as previously described [50]. This assay quantifies the amount of malonaldehyde (MDA) formed as a result of lipid peroxidation and involves reacting samples with 2-thiobarbituric acid (TBA) under high temperatures (90-100°C) and acidic conditions. TBA reacts with a MDA to produce a stable adduct that can be quantified spectrophotometrically.

Blood pressure

A validated automated A&D Medical UA-767 BP monitor (A&D medical, San Jose, CA, USA) was used to measure BP according to Grassi., *et al*. [51]. Three readings of blood pressure were taken before and after each session and the average was calculated.

Dietary Analysis

A record of all food and beverage intake was monitored over the study period to assess compliance with the dietary instructions given and also to determine whether nutritional intake was different between groups. Three-day dietary records were provided to each participant with standard instructions on how to complete the record. Participants were instructed to estimate servings of foods using household measurements as described in national dietary guidance documents as previously described [52]. Diet records were assessed using Windiets™ (2011) for windows (Robert Gordon University, Aberdeen) [53] and were analysed for macronutrient, micronutrient and caloric intake.

Statistics

All data are expressed as mean standard deviation. Data were analysed using the SPSS statistical program (version 21.0 for Windows; SPSS, Inc., Chicago, IL). Data collected pre and one-week post-consumption of PJ or placebo were compared and analysed by a series of paired t-tests. Significance levels were determined by p-value of ≤ 0.05. In addition, Spearman's correlations were used to assess the possibility of an association between the change in Cortisol/ cortisone ratio and the change in blood pressure for basal and post exercise values.

Results

Participants were recruited by email moderator advertisement at Queen Margaret University, Edinburgh. Eligible participants were aged between 18-37 years with a body mass index between 20.1 and 29.5 kg/m^2. In this randomised, placebo controlled, parallel study,

all 24 volunteers (12 males and 12 females) have completed the study and each consumed either 500 mL/day of Pomegreat Pure® pomegranate juice or placebo for a period of 1 week. There were no statistically significant differences observed in mean age (years); height (m); weight (kg), body mass index (kg/m^2) or waist circumference (cm).

	Treatment Group (n = 12)		Placebo Group (n = 12)	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Age (years)	22.1 ± 4.6	20 - 34	22.8 ± 5.1	18 - 37
Weight (kg)	79.7 ± 12.8	62.7 - 105.2	70.6 ± 10.5	55.8 - 90.3
Waist circumference (cm)	89.6 ± 13.1	71 - 117	82.7 ± 11.6	68 - 110
Body mass index (kg/m^2)	26.9 ± 3.8	20.6 - 29.5	25.3 ± 3.7	20.1 - 29.4

Table 1: *Anthropometric characteristics of treatment (n = 12) and placebo (n = 12) participants. Results are expressed as mean values ± standard deviation (SD) and range.*

One week of PJ consumption was associated with a significant reduction in both systolic and diastolic blood pressure compared with the placebo group. SBP was reduced from 136.7 ± 11.7 to 131.8 ± 8.8 mmHg ($p = 0.007$) and 158.8 ± 15.8 to 148.1 ± 12.3 mmHg ($p < 0.01$), pre and post exercise, respectively. Diastolic blood pressure decreased from 86.3 ± 10.6 to 82.5 ± 6.8 mmHg ($p = 0.04$) and 103.1 ± 12.5 to 93.9 ± 11.5 mmHg ($p = 0.001$), pre and post exercise, respectively.

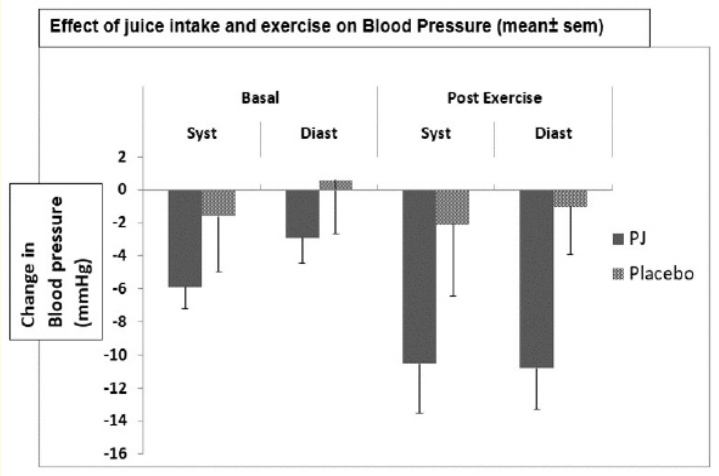

Figure 2: *: Effect pomegranate juice and placebo intake on SBP and DBP pre and post exercise in healthy volunteers.*

Urinary levels of TBARS were significantly reduced, from 31.2 ± 10.6 to 26.5 ± 9.8 MDA µmole/day ($p = 0.035$) following 1 week of pomegranate juice consumption. There were no significant changes observed in markers of lipid peroxidation or blood pressure following the placebo arm of the study.

		Treatment Group (n = 12)		Placebo Group (n = 12)	
		Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
SBP	Baseline	136.7 ± 11.5	158.8 ± 15.8	130.1 ± 14.1	144.5 ± 11.7
Post Intervention		131.8 ± 8.8 *	148.1 ± 12.3**	131.7 ± 13.2	145.1 ± 19.9
DBP	baseline	86.3 ± 8.1	103.1 ± 12.5	85 ± 11.8	92.1 ± 12.6
Post Intervention		82.5 ± 6.8+	93.9 ± 11.5	84.4 ± 9.4	93.3 ± 10.5

Table 2: *Systolic and Diastolic blood pressure (mmHg), pre and post exercise of participants consuming 500 ml/day PJ for 1-week (n = 12) and from a placebo group (n = 12).*

*Statistical significance within each group based on paired t-test: *P = 0.01, **P < 0.01, ‡P = 0.001, + P=0.04. Results are expressed as mean values ± SD, where n = 12.*

Urinary free cortisol was reduced from 179.4 ± 53.2 to 125.6 ± 43.5 nmole/24h which was significant (p = 0.042). In addition, there was a statistically significant increase in urinary free cortisone: from 112.2 ± 40.4 to 187.6 ± 90.2 nmole/24 h (p = 0.045). Calculation of the cortisol/cortisone ratio in urine were found to be significantly decreased (p = 0.009) from 1.6 ± 1.1 to 0.67 ± 0.55 following one week of pomegranate juice intake (see Table 3 and figure 3). Spearman's correlations were run between the Ratio of cortisol/cortisone and change in SBP (SBP Difference) and DBP (DBP Difference) in pre and post exercise of the pomegranate group.

	Treatment Group (n = 12)		Placebo Group (n = 12)	
Parameter	Baseline	After 1-week	Baseline	After 1-week
TBARS (µmole/day)	31.2 ± 10.6	26.5 ± 9.8*	27.1 ± 8.4	28.7 ± 10.7
Free Cortisol (nmole/day)	179.4 ± 53.2	125.6 ± 43.5*	166.6 ± 71.5	191.2 ± 93.7
Free Cortisone (nmole/day)	112.2 ± 40.4	187.6 ± 90.2*	125.5 ± 49.5	136.4 ± 53.8
Ratio: Cortisol/cortisone	1.598 ± 1.1	0.669 ± 0.55**	1.33 ± 0.44	1.41 ± 0.48

Table 3: *Glucocorticoids; free cortisol and cortisone (nmoles/24 h) and thiobarbituric acid-reactive substances (TBARS) (µmole/day) in urine of participants consuming 500 mL PJ daily for 1-week (n = 12) and from a placebo group (n = 12).*

*Statistical significance within each group based on paired t-test: * P < 0.05 and **P < 0-01. Results are expressed as mean values ± standard deviation (SD), where n = 12.*

Urinary free cortisol was reduced from 179.4 ± 53.2 to 125.6 ± 43.5 nmole/24h which was significant (p = 0.042). In addition, there was a statistically significant increase in urinary free cortisone: from 112.2 ± 40.4 to 187.6 ± 90.2 nmole/24 h (p = 0.045). Calculation of the cortisol/cortisone ratio in urine were found to be significantly decreased (p = 0.009) from 1.6 ± 1.1 to 0.67 ± 0.55 following one week of pomegranate juice intake (see Table 3 and figure 3). Spearman's correlations were run between the Ratio of cortisol/cortisone and change in SBP (SBP Difference) and DBP (DBP Difference) in pre and post exercise of the pomegranate group.

The data showed that the following pomegranate juice intake, there was a negative correlation between the ratio of free cortisol/cortisone and the drop in blood pressure.

		Δ SBP- Basal	Δ DBP- Basal	Δ SBP Post	Δ DBP Post		
Ratio Cortisol/cortisone	Correlation Coefficient	-0.347	-0.231	-0.490*	-0.573**		
	Sig. (2-tailed)	0.133	0.326	0.028	0.008		
	N	12	12	12	12		

Table 4: *Spearman's correlations between the cortisol/cortisone ratio versus the drop in SBP and DBP(Δ change) at basal and post intervention group. N = 12.*

*** Correlation is significant at the 0.01 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

Body Mass Index and Waist Circumference

There was no significant change in either BMI or WC in both study groups after the 7 days' intervention period. (BMI- Intervention: 26.72kg/m^2 - 26.85 kg/m^2 p = 0.278, Control: 25.67kg/m^2 - 25.61 kg/m^2 p = 0.509.) (WC- Intervention: 89.8 cm - 89.5 cm p = 0.810, Control: 82.6 cm - 82.7 cm). The analysis of the food diary during the phases of our study showed that there was a comparable intake of total energy and macronutrients in both the PJ and placebo groups suggesting that their food intake did not influence the physiological and biochemical parameters studied.

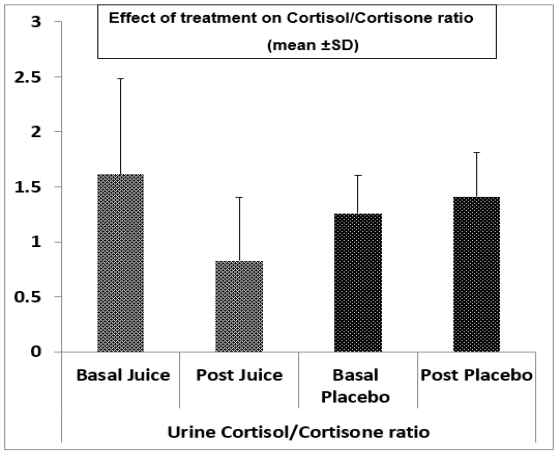

Figure 3: *Effect pomegranate juice and placebo intake on the 24hour urinary cortisol/cortisone ratio in healthy volunteers.*

Discussion

The present study was conducted to investigate the effect of polyphenol-rich pomegranate juice on the cardio-metabolic and oxidative stress effects in healthy male and female overweight participants following an acute bout of moderate intensity exercise. Daily consumption of PJ for 1 week significantly reduced lipid peroxidation (measured as MDA) post-exercise. The substrate used, MDA, is known as a marker of oxidative stress as it is a product of the oxidative degradation of polyunsaturated lipids. In contrast to this, those individuals drinking placebo showed no significant change in their levels of lipid Peroxidation. This therefore highlights that pomegranate juice did produce a reduction in oxidative damage of lipids which in turn shows a reduction in levels of oxidative stress. Oxidative stress has been linked to a growing list of conditions in which a more preventative approach is desirable [54].

Two previous studies have shown a reduction in lipid peroxidation. Research into polyphenols in red fruit juice concluded similar results relating to oxidative stress. For example, patients undergoing haemodialysis and due to their conditions were deemed to be at higher risk of many conditions related to oxidative stress. Following the consumption of 200 ml/day of red fruit juice containing a high level of polyphenols for 4 weeks, researchers found concentrations of the lipid Peroxidation marker MDA were significantly lowered ($p = 0.001$) [35]. A similar study also looked at a heavy smokers group over the age of 50 years who also had increased risk of oxidative stress and involved a randomized double blind cross over trial. They were given a polyphenol rich grape seed extract (in tablet form) for 4 weeks and the results showed that the supplements significantly improved low density lipoprotein cholesterol resistance to oxidation by using TBARS assays [55]. Another study used green tea extracts showed similar results in rats after being treated with green tea extracts for 30 days and were found to have a significantly reduced level of MDA post the intervention period [56].

The results of participant's blood pressure and heart rate measures show promising links between pomegranate juice supplementation and an improvement in general exercise performance. Those in the intervention group had a significant decrease in systolic blood pressure prior to undertaking exercise after one week of drinking the juice whereas the control group showed no such significant decrease. In addition, the pomegranate juice appeared to significantly lower the heart rate of individuals after the 30 minutes of exercise (data not shown), whereas the control group did not show a significant reduction. Similar studies using green tea polyphenols have concluded comparable results with overall reduction in blood pressure (both systolic and diastolic) after consumption of green tea [57,58] and polyphenols found in red wine caused a reduction in overall blood pressure in normotensive subjects [59].

Cortisol is regarded to be one of the important factors in regulating blood pressure, and this has been highlighted in several cases of hypothalamic pituitary adrenal axis conditions, and chronic excessive activation of the glucocorticoid receptor (GR) is known to induce obesity, insulin resistance, glucose intolerance and hypertension [41]. Glucocorticoids exert a direct effect on the heart and blood vessels via 11β-HSD enzymes type 1 and 2. Increased 11β HSD-1 activity is implicated in the development of the metabolic syndrome, type 2 diabetes and obesity, and identifying dietary constituents that can modulate 11β HSD-1 activity could lead to novel methods of preventing CVD and associated risk factors. The association between excess cortisol and various parameters of the metabolic syndrome, including hypertension and insulin resistance, has now been increasingly recognised [42,43]. One of the main objectives of this study was to investigate whether the drop in blood pressure might be caused by inactivation of 11ß-HSD1 enzyme which converts cortisone to the active steroid, cortisol. Plasma cortisol is an important factor in blood pressure regulation and it can also exert negative effects on the cardiovascular system at an autocrine level. There are 2 isozymes of 11ß-HSD that catalyse the interconversion of active cortisol and inactive cortisone. We have shown that PJ intake has the potential of inhibiting 11β-HSD type1 enzyme activity as evident by the reduction in the cortisol/cortisone ratio in both urine and saliva [60] dark grape paper. The role of this enzyme in hypertension has been previously reported, for example in the syndrome of apparent mineralocorticoid excess [61]. Our results showed that there was a negative correlation between the ratio of cortisol/cortisone (indicative of 11ß-HSD1 enzyme activity) and the difference in SBP and DBP pre and post exercise only for those who consumed the pomegranate juice.

Previous research has suggested that antioxidants can protect human cells within the body against damage that can cause diseases such as CVD, diabetes, cancer and other illness and in healthy young and older people [33,62-64] and in mice [65]. This study was carried out as a pilot crossover placebo controlled design to investigate the effects of increasing antioxidant consumption on exercise-induced oxidative stress in healthy volunteers. Participants consuming the pomegranate juice high in antioxidants for a week increased the urinary total antioxidant content levels and significantly decreased lipid peroxidation as measured by the TBARS assay following the juice intake. Zern and colleagues [66] have also reported a cardio protective effect for grape juice in Pre- and Postmenopausal women by lowering plasma lipids and ROS. The body generally has sufficient stores of antioxidant defense to deal with the free radicals and allow the cells to function normally. However, when it is exposed to many factors that cause oxidative stress such as tobacco smoke and other air pollutants (motor vehicles), combined with exercise that is known to induce oxidative stress, the production of these free radical's increases.

This may lead to the defense system working ineffectively causing cell damage, especially if there is a deficiency of antioxidants [67]. As suggested by previous evidence, it is believed that people that partake in regular exercise have a higher antioxidant capacity [68-70]. In our study, the marker of oxidative stress, TBARS was significantly reduced in the group consuming the pomegranate juice following the exercise bout. However, the group who drank the placebo did not show an improvement in TBARS levels.

There is still a lack of evidence that exercise-induced oxidative stress and antioxidant supplementation can affect sporting performance [67]. In 1978, Dillard., *et al*. [71] were the first to discover a link between exercise and oxidative stress. They found that there was an increase in exhaled pentane levels after cycling for one hour. Pentane is a possible by-product of oxidative damage. This evidence has been supported by several studies carried out since then. A study by Bejma and Ji [72] involving both young and old rats found that there was an increase in reactive oxygen species (ROS) production due to exercise in both groups. However, it was discovered that heart ROS production was increased in the older rats only. This suggests that the effect on heart oxidative stress may be age related. As we get older it is believed that the body defence system works less efficiently against the production of ROS. The rate of lipid peroxidation in the muscle increases as we age and evidence has shown that as the antioxidants are not sufficient enough to cope with this increase. This suggests that the elderly is more prone to cell damage due to oxidative stress and therefore may benefit from an increase in antioxidants in the diet [70]. Indeed, Jackson and McArdle [73] reported age-related changes in skeletal muscle reactive oxygen species generation and adaptive responses to reactive oxygen species. By performing regular exercise training, it is thought that oxidative stress capacity can be increased, along with enzymatic antioxidant defence against free radicals. Although, training does stimulate the release of antioxidant enzymes, it is thought that vitamin E is needed by the muscle during intense exercise [70]. However, a study was carried out investigating antioxidant response during exhaustive exercise. This study suggested that intense exercise over a prolonged period of time can cause such a rapid increase in the production of free radicals that can be too intense for the antioxidant defence system and thus result in tissue damage [2]. Garcia- Alonso., *et al*. [74] supported this hypothesis when they looked into the short term effect of consuming a juice high in polyphenols and also discovered that lipid peroxidation was reduced. Another study carried out by Morillas-Ruiz and colleagues [75] looked into the effect of polyphenolic antioxidants on exercise- induced oxidative stress and reported that consuming high levels of polyphenols whilst exercising had a positive effect on markers of oxidative stress.

Our study had some limitations for example, the small sample size. A larger sample size would be essential. Due to the small sample and the method of recruitment, the age range of participants was low, with only one volunteer being over the age of 25. However, a good range of other attributes of the participants including BMI, WC, and blood pressure. The design of the study was planned to last foe only one week which is a very short timescale due to time restrictions of the study. The lack of time also affected the amount of samples that could be collected and analysed which meant no urine samples were collected prior to the initiation phase to provide a basal measurement of antioxidant capacity and total Phenolic levels. The protocol was constructed for participants to drink 500 mL of pomegranate juice daily, which showed significant benefits. However, this might be impractical, and future studies could perhaps involve the same process but with the pomegranate juice in a smaller quantity, or a different form (tablet or capsule) to enable this to become promoted as a realistic health supplement. Some potential participants were embarrassed by the concept of collecting 24hour urine samples and thought it would be too time consuming, restrict their movement and social life. Several volunteers had social events due to the festive period which meant that they were unable to exercise or consume the required amount of juice for that day. Although participants were given a list of foods rich in polyphenols to restrict their intake during the study, this may not have been precisely followed.

Conclusions

Our results suggest that pomegranate juice consumption moderates exercise-induced oxidative stress and reduces systolic and diastolic blood pressure. This may be due to the inhibition of 11 βHSD type 1 activity as evidenced by the reduction in the cortisol/cortisone ratio or other mechanisms yet to be investigated. The results as well as those of similar research in this area provide a definitive need for these benefits to be explored further. With cardiovascular disease and various cancers being the leading cause of death in the U.K, preventative measures with diet intervention showing signs to be the key to reduce CVD risk.

Conflict of interest statement

The authors have declared no conflict of interest and nothing to disclose. EASAD was responsible for designing, conducting the research and statistical analysis and preparing the manuscript. CT provided essential reagents and assisted with antioxidant analyses. GG has recruited the volunteers and performed the anthropometrical measurements.

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Volume 4 Issue 6 August 2016