

The Effect of Dark Grape Juice Consumption on Exercise-Induced Oxidative Stress in Healthy Adults Aged 41 to 60 Years

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Abstract

Background: It is now well established that fruit juices are rich in antioxidants that are beneficial to our health. This study was designed to investigate the effect of dark grape juice intake rich in antioxidants on oxidative stress induced by exercise in 41 to 60 years old.

Method: Subjects were split into group A (n = 12 exercisers) and group B (n = 10 non exercisers). The study was conducted using a crossover randomised single blind placebo controlled design: Week 1: 24 hour urine collection (baseline) was done and anthropometric, blood pressure and diet diaries were recorded. Week 2: volunteers consumed 1 liter of placebo (sweetened water in dark container) or 1 litre of dark grape juice per day for 1 week and followed usual exercise regime and 24 hour urine was collected at the end of the week. Week 3 was the washout and free for all participants. Week 4: the exact same format was followed as in the previous week. However, those who consumed placebo switch to grape juice and vice versa. Another 24 hour urine sample was collected. A 3-day food diary was also kept in week 2 and 3. The total phenolic content, ferric reducing antioxidant power (FRAP) and TBARS were analyzed in all urine samples using established methods that were checked using quality control samples.

Results: A significant reduction in systolic BP following the DG juice in the exercisers (from 136 ± 8.7 to 132.2 ± 8.9 mmHg, $p = 0.03$) and the non-exercisers (from 137.7 ± 10.1 to 135.2 ± 9.9 mmHg, $p = 0.04$), but the reduction was more marked for the exercisers. Diastolic BP was only significantly reduced in the exercisers ($p = 0.05$). There was no significant change in weight, BMI and heart rate. There was a significant increase in the urine total phenolics (from 400.8 ± 101.1 to 479.5 ± 107.9 mg GAE/day, $p = 0.022$) and FRAP (from 7.66 ± 2.97 to 9.27 ± 2.98 mmole Fe (ii)/day, $p = 0.026$) levels, but a decrease in TBARS levels (from 31.2 ± 9.12 to 24.4 ± 7.6 μ mole/day, $p = 0.028$) in the exercisers group after DG juice intake. The total phenolics excretion in the non-exercising group did also increase significantly ($p = 0.04$), however, there was no significant increase in FRAP or decrease in TBARS ($p = 0.367$ and $p = 0.07$ respectively). No significant changes were obtained in all parameters studied following the placebo intake and between all the total 24 hour urine volumes collected from both groups.

Conclusion: In individuals aged 41 to 60 years old who exercise regularly, intake of dark grape juice have significantly increased the total phenolic and FRAP urinary levels and caused a significant decrease in one oxidative stress marker. However, for the aged-matched non-exercisers, it produced an increase in total phenolic but did not appear to have a significant effect on oxidative stress markers.

Keywords: Grape juice; Polyphenols; CVD; Oxidative stress; Blood pressure

Abbreviations: BMI: Body mass index; BP: Blood pressure; CVD: Cardiovascular disease; DNA: Deoxyribonucleic Acid; DG juice: Dark grape juice; GAE: Gallic acid equivalents; FRAP: Ferric reducing anti-oxidant power; LDL: low density lipoprotein; MDA: Malonaldehyde; ROS: Reactive Oxygen Species; RNS: Reactive nitrogen species; TBARS: Thiobarbituric acid reactive substances

Introduction

There is a vast amount of evidence to show that antioxidants are beneficial to our health and it is thought that they could help to counteract the effects of oxidative stress and boost the endogenous anti-oxidant defence of the body [1-5]. Oxidative stress occurs when there is an imbalance in biological systems causing the production of reactive oxygen species known as ROS. Highly reactive atoms which are called free radicals can then form and these react with the cell organelles and biomolecules and are capable of causing the cell to malfunction or die. Oxidative stress causes damage to DNA, lipids and proteins within the body. Increased production of ROS is linked to many diseases, such as cardiovascular disease (CVD) and cancer [6,7]. Powers and colleagues have published several detailed reviews and articles on the roles of ROS and RNS within muscle and other tissues during exercise and concluded that muscular exercise and long periods of muscle disuse promote ROS production in skeletal muscle fibres [8-10]. The body has many defence systems that work against these free radicals to counteract the damage caused to the cells. One of these involves both enzymatic and non enzymatic antioxidants that participate in a defence system within the body. The main antioxidant enzymes include superoxide dismutase, glutathione peroxidase and catalase. The main non enzymatic antioxidants are polyphenols, ascorbate, tocopherols and others [11]. These systems are not completely efficient as the body cannot synthesise non enzymatic antioxidants so they must be provided in the diet [12].

Flavonoids are the largest group of polyphenols found in grapes and pomegranates and recent studies have shown that there are many beneficial effects to human health following the consumption of grapes, grape products, pomegranates and other polyphenol-rich fruits [1]. These phytochemicals can act as antioxidants, anti-inflammatory and being cardio and cerebrovascular protectors. All of these effects would help in the treatment and prevention of CVD, diabetes, obesity and some cancers [2,13-16]. The prevalence of CVD is ever increasing globally due to changes in lifestyle such as a high fat diet, processed food and lower physical activity levels that are key factors in the development of cardiovascular diseases [17].

Polyphenols are the most abundant antioxidants in our diet. Polyphenols are found in plants and the main sources in our diet are berries, tea, beer, grapes, pomegranates, red wine, dark chocolate, coffee, nuts, fruit, fruit juices and vegetables [12]. When an individual participates in exercise, catabolic and anabolic processes take place within the muscle during and after the exercise session. Free radicals are thought to play a major role in this as they are released into the muscle and this is thought to trigger a metabolic response. Jacob and Burri. [7] stated that ROS seem to play a part in exercise-induced muscle membrane injury and also alter the activity of certain enzymes. The degree of damage to cells due to exercise would depend on the balance of oxygen uptake and antioxidant balance. It is clear that antioxidants play a beneficial role in our health. However, there is still lack of evidence to give a clearer understanding of the effect of antioxidant intake of polyphenols when looking at exercise and oxidative stress [18]. In animal research, a group found that grape seed extract intake improved adipokine imbalance and oxidative stress markers in hamsters following high-fat diet-induced obesity [19]. Recently, Georgiev [20] and colleagues published an interesting review on the recent advances and uses of grape flavonoids as nutraceuticals, and it is thought that grape and grape products can exert their multi-organ protective functions due to the presence of abundant quantities and varieties of bioactive compounds such as flavonoids. The specific chemical structure of flavonoids can facilitate the reduction of oxidative stress through several mechanisms (for example, flavonoids can act both as preventive and chain-breaking antioxidants [21], or they can act as metal chelators and enzyme inhibitors as explained by Pietta [22]). Therefore, the aim of this pilot study is to find out if the intake of dark grape juice (rich in polyphenols) affects oxidative stress induced by exercise in a cohort of 41 to 60 years old, and whether there is a difference between those who exercise regularly and sedentary individuals.

Methods

The project was granted ethical approval by the Queen Margaret University Ethics committee. The study followed a randomised placebo controlled cross over design. The aim of this study is to find out if polyphenols in dark grape juice affect oxidative stress induced by exercise in 41 to 60 years old. Specific objectives were to find out if drinking dark grape juice for one week can influence the levels of the

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urinary total phenolics, FRAP and an oxidative stress marker (TBARS) in exercisers when compared to non exercisers. Another objective is to investigate the effect of dark grape juice consumption on Blood pressure and heart rate in this set age group.

Participants

A total sample size of 22 individuals aged 41-60years were recruited for a four week study. Subjects were recruited by various methods of communication. Posters were displayed in health clubs, e-mails were circulated at the university and through personal contacts. An information sheet was given to participants and they were also given the chance to ask any questions they had. The individual was assessed for suitability for the study. If the individual met the criteria (see figure 1) and they were willing to participate then they were asked to complete the required consent form.

Study protocol

The subjects were split into group A (n = 12) and group B (n = 10). Group A was made up of those that partake in regular aerobic exercise and Group B was made up of a control group of individuals who were less physically active. Regular exercise was classed as doing aerobic exercise at least three times a week. In week one, participants were required to collect a 24 hour urine sample (baseline) and follow their usual routine (they were briefed on the correct method to collect the 24 hour urine sample). During week two, individuals were required to consume either one litre of sweetened water in dark container containing the same caloric energy as the grape juice (placebo) or consume (through out the day) 1 liter of organic Concord dark grape juice containing 1450 mg/L polyphenols (intervention) per day for 7 days and collect a 24 hour urine sample at the end of that week including the exercise day. The participants followed their normal exercise routine. There was then a washout period of 7days and then the final week of the study followed the exact format as the week before. However, those who consumed placebo switched to grape juice and vice versa (Figure 1). Participants were instructed on how to keep a food diary and this was kept over three days over week 2 and 4 each. An information sheet was given to all volunteers on foods high in polyphenols and asked to restrict their intake during the study. They were advised that they can continue to drink their usual intake of water, tea and coffee throughout the study and should avoid alcohol consumption 24 hours prior to collecting urine samples. The urine samples were stored at room temperature until collected. The samples were coded according to the participant's number for anonymity. The total volume of each sample was recorded and two aliquots from each collection were frozen at -20°C until required for analysis. Win Diets Research software programme (Robert Gordon University, Aberdeen, UK) which can analyze multiple diets in surveys with output in spreadsheet format was used to assess the participant's food diaries.

Laboratory measurements

The total phenolic content of the urine sample was analysed following a modification of Singleton and Rossi [23] method. This allows the total phenols to be determined using Folin and Ciocalteu reagent (FC). A blue colour is produced from reducing yellow heteropolyphosphomolibdate-tungstate anions. Gallic acid was dissolved in distilled water and used to construct the standard curve and the urine samples were diluted with distilled water (1:5 or 1:10) before the assay. Briefly, 50 µL of standard or diluted urine samples were incubated with 2.5 mL FC reagent after 5 minutes, 1.75 mL of sodium carbonate solution (0.2 mole/L) was added, the tubes were mixed and incubated in the dark at room temperature for 2 hours, then read spectrophotometrically at 765 nm. The antioxidant power was determined using the FRAP assay as described by Benzie and Strain (1996). This method involves the ability of a solution to reduce ferric-2,4,6-tri-2-pyridyl-s-triazine (TPTZ) complex (Fe^{3+} TPTZ) to the ferrous form (Fe^{2+}). An intense purple colour is produced when ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex is reduced to the ferrous form (Fe II). There is excess Fe^{3+} in the FRAP assay and this is used as the limiting factor of the Fe^{2+} -TPTZ to measure the reducing ability of the sample. Test conditions favour reduction of the complex and thereby colour development, provided that a reductant (antioxidant) is present. Briefly, 10 µL of standard or diluted sample were incubated with 4 mL of FRAP reagent, the tubes were then mixed, incubated at 37°C for 5 minutes and the absorbance read at 595 nm. Thiobarbituric reactive substances (TBARS) assay was performed for the assessment of oxidative stress levels in urine samples as previously described [24]. 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. Briefly, 100 µL of urine sample or standard were incubated

with 1mL TBARS reagent and the tubes were incubated at 98°C for 30 minutes and then read at 542 nm. All reagents and chemicals were purchase from Sigma Aldrich, Poole, UK.

Statistical Analysis

Data was statistically analysed using excel and the statistical package for the social sciences (SPSS, version 17). The mean and standard deviation of all data was calculated. SPSS was used to perform t-tests to compare data. Repeated measure analysis of variance (2-way ANOVA) was planned to be used to analyze all parameters studied to find out any significant changes between the basal, placebo and intervention for each group of volunteers. All variables were first tested for normal distribution to assess if a parametric test could be carried out. In order to evaluate the variation between the three groups two-way ANOVA was thought to be ideal, however not all parameters were normally distributed making the ANOVA results inaccurate. As a result a student 2-tail paired t-test was employed to compare changes from baseline to both DG juice and placebo and between the two interventions acknowledging that the probability of making a type II error increases from 5 to 14%. Descriptive statistics and a 2-paired t-test were performed using SPSS statistics (version 17.0), while Excel was used to calculate total phenolics, TBARS and FRAP concentrations and for the confirmation of the t-test results. Values were expressed as the mean ± SD and significance by p-value ≤ 0.05 [25].

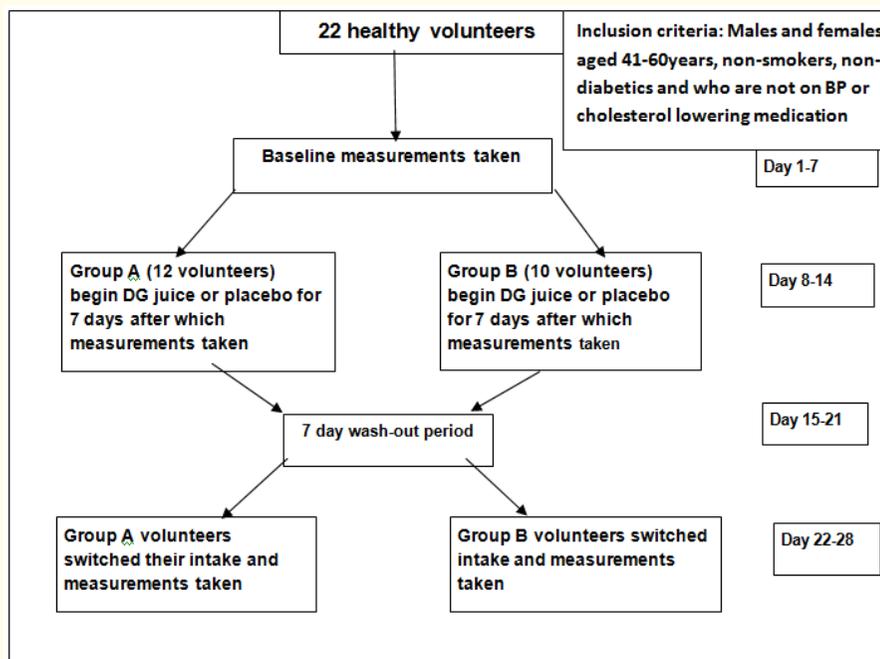


Figure 1: A flow diagram of the randomised cross-over study protocol and design.

Results

Participants were successfully recruited aged between 41 to 60 years (n = 24) with a BMI of less than 30kg/m² (females) and 35 kg/m² (males), however two participants dropped out for personal reasons. The final number of participants was 22 which consisted of 12 regular exercisers (Group A) and 10 control non-exerciser participants (group B). Table 1 shows the results of anthropometric and BP characteristics for all volunteers before (baseline) and post intake of DG juice for one week. The data showed a significant reduction in systolic BP following the DG juice in the exercisers (systolic BP from 136 ± 8.7 to 132.2 ± 8.9 mmHg, p = 0.03) and the non-exercisers (from 137.7 ± 10.1 to 135.2 ± 9.9 mmHg, p = 0.04), but the reduction was more marked for the exercisers. Diastolic BP was only significantly reduced in the exercisers (p = 0.05). Following the placebo intake, Systolic and diastolic BP in exercisers were not significantly

changed (from 135.7 ± 7.9 to 136.3 ± 9.1 ; $p = 0.458$, and 77.9 ± 7.5 to 78.4 ± 8.1 mmHg; $p = 0.786$ respectively). Similarly, systolic and diastolic BP in non-exercisers varied from 137.9 ± 11.2 to 138.8 ± 11.8 ($p = 0.772$) and 83.1 ± 8.3 to 82.8 ± 7.8 mmHg ($p = 0.984$) respectively. There were no significant changes in weight, BMI and heart rate in all volunteers post DG juice or placebo.

	Exercisers Group (n = 12)		Non-Exercisers (n = 10)	
	Basal	Post DG Juice	Basal	Post DG Juice
Age (years)	49.5 ± 7.1		51.3 ± 7.6	
Body mass index (kg/m ²)	25.9 ± 3.4	26.1 ± 2.9	27.3 ± 4.4	26.9 ± 3.1
Weight (kg)	79.9 ± 8.8	80.2 ± 10.2	81.3 ± 11.2	80.8 ± 9.3
Hear rate (bpm)	70.5 ± 12.1	68.2 ± 7.4	72.7 ± 13.5	68.2 ± 10.1
Systolic BP (mmHg)	136 ± 8.7	132.2 ± 8.9**	137.7 ± 10.1	135.2 ± 9.9*
Diastolic BP (mmHg)	78.6 ± 8.8	75.2 ± 7.6*	82.6 ± 8.6	81.2 ± 7.7

Table 1: Anthropometric and BP characteristics of exercisers (n = 12) and non-exercisers (n = 10) participants. Results are expressed as mean values ± SD. Significance: * $p = 0.04$, and ** $p = 0.03$.

There was no significant difference between the total volume excreted of the basal, the placebo and the intervention. However, there was a slight increase following the placebo compared to the intervention. As the urine collections were not carried out in a controlled environment the accuracy of the results may be affected. Figure 2 shows that the urinary total phenolic content mean for all participants during the intervention (456.7 ± 137.4 mgGAE/day, n = 22) was higher than the mean during the placebo (346.9 ± 66.5 mgGAE/day, n = 22), and this was a significant increase ($p = 0.026$). A comparison of urinary total phenolics between exercisers and non-exercisers indicates that the increase in total phenolics for exercisers: baseline against intervention, from 400.7 ± 101 to 479.5 ± 107.9 mgGAE/day ($p = 0.045$); baseline against placebo, down to 358.1 ± 95.7 mgGAE/day ($p = 0.142$); placebo against intervention, from 358.1 ± 95.7 to 479.5 ± 107.9 mgGAE/day ($p = 0.022$). For non-exercisers: baseline against intervention, from 322.1 ± 170.5 to 412.5 ± 167.7 mgGAE/day ($p = 0.039$); but baseline against placebo was not significant ($p = 0.781$), and placebo against intervention was just significant, from 339.7 ± 42.9 to 412.5 ± 167.7 mgGAE/day ($p = 0.05$).

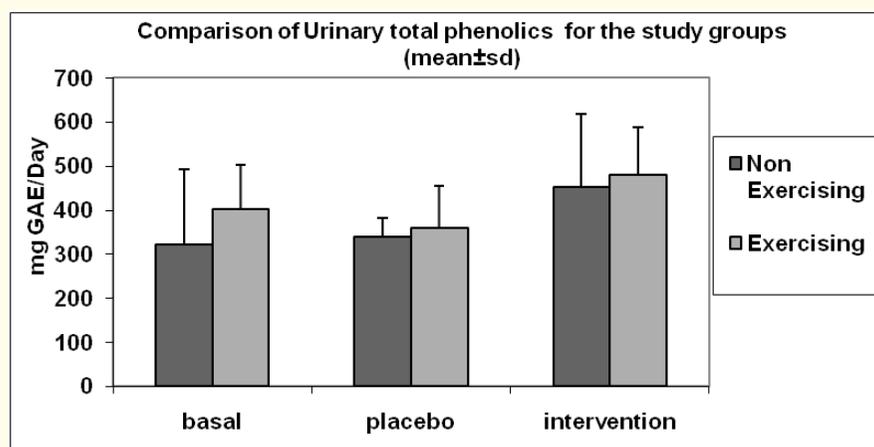


Figure 2: Comparison of urinary total phenolics between exercisers and non-exercisers. The increase in total phenolics for exercisers: baseline versus intervention, $p = 0.045$; baseline versus placebo, $p = 0.142$; placebo versus intervention, $p = 0.022$. For non-exercisers: baseline versus intervention, $p = 0.039$; baseline versus placebo, $p = 0.781$; placebo versus intervention, $p = 0.05$.

Figure 3 shows a comparison of urinary FRAP results between exercisers and non-exercisers. The increase in FRAP levels for exercisers: baseline against intervention from 7.66 ± 2.97 to 9.27 ± 2.98 mmole Fe (ii)/day ($p = 0.026$); baseline against placebo was not significant, from 7.66 ± 2.97 to 8.25 ± 3.32 mmole Fe (ii)/day ($p = 0.386$) and placebo against intervention from 8.25 ± 3.32 to 9.27 ± 2.98 mmole Fe (ii)/day ($p = 0.258$). For non-exercisers: No significant change between baseline, placebo and intervention levels was found, baseline against intervention from 6.69 ± 2.68 to 7.8 ± 2.96 mmole Fe(ii)/day ($p = 0.367$). In general, the mean of the antioxidant power in the exercising group for both the placebo and the intervention was higher in comparison to the non-exercisers group. Results of lipid peroxidation as measured by the TBARS assay are shown in figure 4. Urinary levels of TBARS were significantly reduced in both exercisers and non-exercisers groups following the DG juice consumption, however the decrease was not significant in the non-exercisers volunteers; baseline against intervention from 29.7 ± 8.87 to 26.5 ± 8.2 μ mole/day ($p = 0.07$). The decrease in TBARS for exercisers: baseline against intervention (from 31.2 ± 9.1 to 24.4 ± 7.6 μ mole/day, $p = 0.02$); baseline against placebo from 31.2 ± 9.1 to 32.3 ± 8.8 μ mole/day ($p = 0.36$); placebo against intervention from 32.3 ± 8.8 to 24.4 ± 7.6 μ mole/day, $p = 0.03$). For non-exercisers: No significant change between baseline, placebo and intervention levels was found.

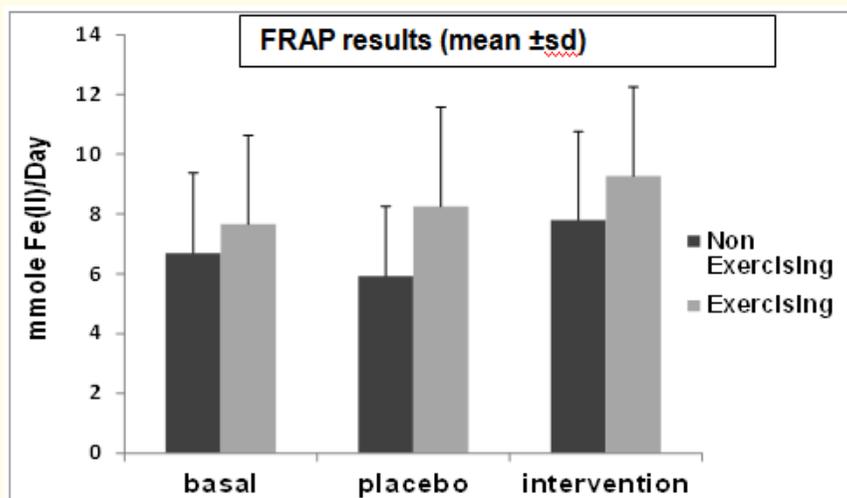


Figure 3: Comparison of urinary FRAP results between exercisers and non-exercisers. The increase in FRAP levels for exercisers: baseline versus intervention, $p = 0.026$; baseline versus placebo, $p = 0.386$; placebo versus intervention, $p = 0.258$. For non-exercisers: No significant change between baseline, placebo and intervention levels was found.

When food diaries were analyzed using Windiets software, the total mean energy content of the participant's food diary during the placebo (2078 ± 424 kcal/day) was not significant from the intake during the intervention (2237 ± 396 kcal/day; $p = 0.078$, $n = 22$). The fat content has increased by 7g during the intervention (87 ± 15.5 g/day) compared to the placebo (80 ± 13.2 g/day) which was not significant ($p = 0.145$). Studying the protein content it appears there was an increase of 1.6g from the placebo (75.6 ± 18.2 g/day) to the intervention of 77.2 ± 12.2 g/day) which was also not significant ($p = 0.609$). The carbohydrate content increased during the intervention compared to placebo (from 289.6 ± 58.8 g/day to 316.5 ± 64.6 g/day) but the increase was not significant due to the high sd. ($p = 0.124$).

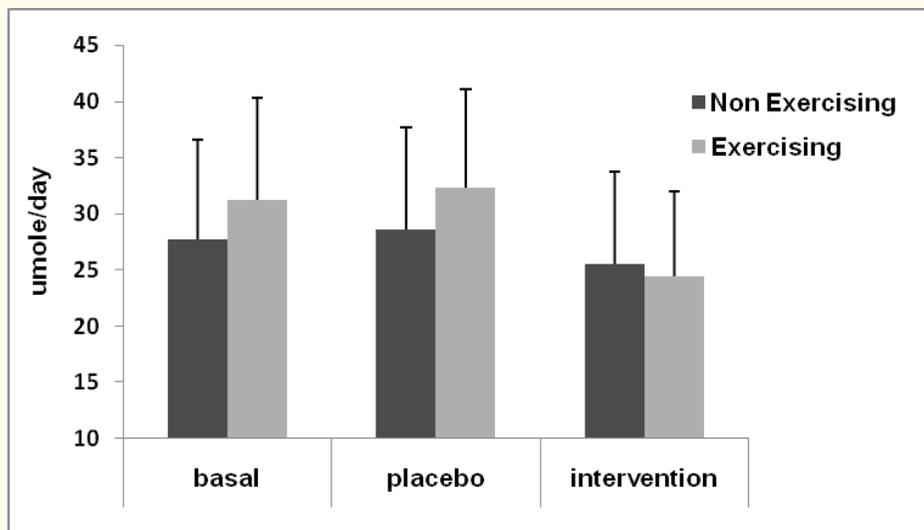


Figure 4: Comparison of urinary TBARS results between exercisers and non-exercisers. The decrease in TBARS for exercisers: baseline versus intervention, $p = 0.02$; baseline versus placebo, $p = 0.36$; placebo versus intervention, $p = 0.03$. For non-exercisers: No significant change between baseline, placebo and intervention levels was found.

Discussion

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 [1,2,4,26] and in mice [27]. 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 exercisers aged 41 to 60 years old compared to non exercisers. Participants consumed a litre of dark grape juice high in antioxidants for a week. Consuming the grape juice increased significantly the urinary total antioxidant content levels, and in particular, in the exercisers group. In contrast, lipid peroxidation was decreased as measured by the TBARS assay following the juice intake. This study also presented the results where the effects of the polyphenols in dark grape juice were investigated as the total phenolics excreted in the urine of exercisers and non exercisers. The total phenolics results for all participants combined increased significantly from the placebo to the intervention which indicates that as a whole the participants have complied by consuming the grape juice or placebo as instructed. When comparing the groups separately it can be seen that the increase was more marked in the exercising group, and this group had higher intake of polyphenol-rich food at baseline. Zern and colleagues [28] have also reported a cardioprotective effect for grape juice in Pre and Postmenopausal women by lowering plasma lipids and ROS. In fact, the majority of the grape health protective properties are attributed to its high content of flavonoids that are not limited to antioxidant, anti-inflammatory, anti-cancer, antimicrobial, neuroprotective and others [20]. The most common flavonoids found in grapes are anthocyanins, flavonols, flavanols and proanthocyanidins [29]. The antioxidant action of flavonoids is mostly attributed to their specific chemical structure in facilitating the reduction of oxidative stress through several mechanisms [21]. *In vitro* studies have shown that flavonoids could act as preventive and chain breaking antioxidants (scavenging superoxide, peroxy and hydroxyl radicals and preventing LDL oxidation), In addition, flavonoids can chelate metals and act as enzyme inhibitors for ROS generation, xanthine oxidase, lipooxygenase, cyclooxygenase and others [22]. *In vivo* studies have also shown that flavonoids can up-regulate the body anti-oxidant defense system and increase uric acid plasma levels and by their conversion to simple phenolic acids which are responsible for the scavenging of free radicals and improving the action of endogenous and other antioxidants [20].

The body generally has sufficient stores of antioxidant defence 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 radicals increases. This may lead to the defence system working ineffectively causing cell damage, especially if there is a deficiency of antioxidants [11]. As suggested by previous evidence, it is believed that people that partake in regular exercise have a higher antioxidant capacity [7,8,10]. In our study, the antioxidant power increased during the intervention in the exercisers this suggests that the antioxidants in the grape juice may have improved the antioxidant power even further. However, the group of exercisers, who are more likely to be affected by oxidative stress, are thought to respond more rapidly to antioxidant supplementation than those who are less likely to be affected by oxidative stress (non-exercisers group) [30]. This could explain the increase in antioxidant power during the intervention in the exercising group. As the participants only consumed the grape juice over a one week period this may have limited the full antioxidant affect. O'Byrne, *et al.* [30] investigated the effect of an antioxidant rich drink on oxidative stress over a two week period. They stated that two weeks may not be long enough for the antioxidants to work efficiently and hence may not be long enough to protect against oxidative damage. They suggested that an eight weeks period to be a more valid time period to allow for any significant results as shown by Marangon, *et al.* [31]. However, any antioxidant effect from the grape juice may be due to the vitamin C content and not the polyphenols. Vitamin C is another antioxidant that is present in the grape juice consumed. As the participants were not given a placebo drink containing the same quantity of vitamin C, this may explain any changes, even if insignificant. On the other hand, Morillas-Ruiz, *et al.* [32] carried out a similar study and gave participants a beverage containing the same amount of vitamin C during both the placebo and the intervention and discovered that the antioxidant effect of the drink could be due to the polyphenols.

There is still a lack of evidence that exercise-induced oxidative stress and antioxidant supplementation can affect sporting performance [11]. In 1978, Dillard, *et al.* [33] 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 byproduct of oxidative damage. This evidence has been supported by several studies carried out since then. A study by Bejma and Ji. [34] 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. But both the above studies had some limitations with regards to methodology. 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 are more prone to cell damage due to oxidative stress and therefore may benefit from an increase in antioxidants in the diet [7]. Indeed, Jackson and McArdle [35] reported age-related changes in skeletal muscle reactive oxygen species generation and adaptive responses to reactive oxygen species. They concluded that during ageing all tissues, including skeletal muscle, demonstrate an accumulation of oxidative damage that may contribute to loss of tissue homeostasis which was due to the reduction of cytoprotective proteins in older people through activation of redox-sensitive transcription factors.

By partaking in 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 [7]. 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 [36]. A study involving grape juice intake in healthy adults [29] showed that the antioxidants in the juice increased antioxidant capacity and low density lipoprotein resistance to oxidative damage. However, it did not have such a large impact on protein oxidation. This was thought to be due to the short time period and certain dietary restrictions. Garcia-Alonso, *et al.* [37] 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 [32] 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. However, a limitation of this study was that no control group was used.

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Daily consumption of dark grape juice for 1 week significantly reduced lipid peroxidation (TBARS measured as MDA) post-intervention. In contrast, the placebo drink showed no significant change in the levels of lipid Peroxidation. This therefore highlighted the fact that grape juice did produce a reduction in oxidative damage to lipids which in turn caused a reduction in levels of oxidative stress. Urinary TBARS is regarded as an important measure to indicate the oxidative damage to lipids and therefore reflects balance between oxidants and anti-oxidants *in vivo*. Several studies have shown a reduction in lipid peroxidation following the intake of food rich in polyphenols. In participants undergoing haemodialysis who were given 200 ml/day of red fruit juice containing a high level of polyphenols for 4 weeks, TBARS assay levels were significantly lower after the period of juice consumption [38]. Another randomized double blind cross over trial looked at heavy smokers who had increased risk of oxidative stress over the age of 50 years. They were given a polyphenol rich grape seed extract (in tablet form) or a placebo for 4 weeks. Results showed that the supplements significantly improved low density lipoprotein cholesterol resistance to oxidation by using TBARS assays [39]. A third study involving the intake of green tea extracts in rats showed similar results. Rats were given green tea extracts for 30 days and were found to have a significantly reduced level of MDA after the intervention period [40].

The analysis of the food diary during the phases of our study showed that there was a comparable intake of total energy and macronutrients suggesting that their food intake did not influence the physiological and biochemical parameters studied. This study was intended to be a pilot intervention, and therefore the number of participants involved in the study is regarded to be low. This may have reduced the accuracy of the results as the participants may not be fully representative of the subject population. In future studies a larger sample size should be recruited to obtain more accurate and significant results. In addition, due to the criteria of the study to recruit suitable participants on a set age group of 41 to 60 years old, it was difficult to find people that were within the required body mass index range and those who were not on any medication or that did not have a health condition that would affect participation. Some potential participants were embarrassed by the concept of collecting 24 hour urine samples and thought it would be too time consuming, restrict their movement and social life. As the study took place over winter and hence a time of many social events many participants reported that this affected their compliance. 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.

There are limitations of the Folin-Ciocalteu method used to measure the total phenolics in the samples. As stated by Roura, *et al.* [41] there are certain compounds present in the urine that can affect the assay such as ascorbic acid and this could have affected the results obtained. The Folin-Ciocalteu method is favored compared to other methods such as Folin-Denis as the results produced are believed to be more reliable. It is thought that the Folin-Ciocalteu is less likely to be affected by interference from non phenolic compounds and provides reproducible results. The method for the FRAP assay was adapted for use with urine samples and was based on the original method by Benzie and Strain [42] which was used with plasma samples. The FRAP assay is simple to prepare, inexpensive and gave reproducible results. The method had to be tested using different concentrations of the urine sample to obtain accurate and reliable results. FRAP assay is intended to measure soluble antioxidants capacity and thus does not accurately reflect the *in vivo* antioxidant capacity. Due to the fact that only urine samples were collected, therefore the FRAP data did not give any information on the enzymatic antioxidant systems. To elucidate this issue, future studies have to collect blood samples in order to estimate the *in vivo* antioxidant capacity.

As antioxidants have a short lifespan and this could have affected the results obtained. A study carried out by Ghiselli, *et al.* [43] looked into the effect of the storage on the antioxidant capacity of the samples in blood cells. They discovered that there was a significant decrease in antioxidant capacity after three days in samples stored at -80°C. Any future studies should consider this when storing and analysing samples. Uric acid has a potent antioxidant activity and could again have affected the results obtained. Gender and metabolic differences can also affect uric acid levels and could be a possible confounding factor of the measurement of antioxidant power. Ascorbic acid and uric acid also can contribute to the antioxidant capacity in biological fluids and can affect the results of assays obtained [44]. In any future studies high performance liquid chromatography (HPLC) might be better used to examine the total phenolic content and antioxidant power excreted in the urine. HPLC can be used to separate different compounds in biological fluids. Roura,

et al. [41] used this method and found that it made a significant difference to the results obtained in term of improving the accuracy of the results and identifying various active metabolites of polyphenols [45,46].

Conclusions

This study has shown that the total polyphenols in urine increased significantly after consuming dark grape juice. There was also a significant increase in antioxidant power in those who exercise regularly after consuming the dark grape juice. More importantly, lipid peroxidation indicator and BP were significantly reduced suggesting a potential benefit in the oxidative stress status for exercisers. In future studies, we recommend that a larger size study, a controlled diet and exercise regime should be used. Blood samples can also be collected to test accurately the enzymatic antioxidant systems and other oxidative stress biomarkers.

Conflict of Interest

The authors have declared no conflict of interest and nothing to disclose. EASAD and HT was responsible for designing and conducting the research and preparing the manuscript. JH collected the samples and performed the urinary FRAP, TBARS and total polyphenols assay.

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Bibliography

1. Hyson DA. "A Review and Critical Analysis of the Scientific Literature Related to 100% Fruit Juice and Human Health". *Advances in Nutrition* 6.1 (2015): 37-51.
2. Tsang C., *et al.* "Intake of polyphenol-rich pomegranate pure juice influences urinary glucocorticoids, blood pressure and homeostasis model assessment of insulin resistance in human volunteers". *Journal of Nutritional Science* (2012): e9.
3. Wahlqvist ML. "Antioxidant relevance to human health". *Asia Pacific Journal of Clinical Nutrition* 22.2 (2013): 171-176.
4. Bjelakovic G., *et al.* "Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases". *Cochrane Database of Systematic Reviews* (2012): 3.
5. Li Y., *et al.* "Serum and dietary antioxidant status is associated with lower prevalence of the metabolic syndrome in a study in Shanghai, China". *Asia Pacific Journal of Clinical Nutrition* 22.1 (2013): 60-68.
6. Halliwell B and Gutteridge JMC. "Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy". *Lancet* 1.8391 (1984): 1396-1397.
7. Jacob RA and Burri BJ. "Oxidative damage and defence". *Clinical Nutrition* 63 (1996): 985-990.
8. Powers SK., *et al.* (2010) "Reactive oxygen species are signalling molecules for skeletal muscle adaptation". *Experimental Physiology* 95.1 (2010): 1-9.
9. Powers SK and Jackson MJ. "Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production". *Physiological Reviews* 88 (2008): 1243-1276.
10. Powers SK., *et al.* "Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle". *The Journal of Physiology* 589.9 (2011): 2129-2138.
11. Cooper CE., *et al.* "Exercise, free radicals and oxidative stress". *Biochemical Society Transactions* 30.2 (2002): 280-285.
12. Hideyuki I., *et al.* "Polyphenol levels in human urine after intake of six different polyphenol-rich beverages". *British journal of nutrition* 94 (2005): 500-509.
13. Ananga A, *et al.* "Manipulation of engineering of metabolic and biosynthetic pathway of plant polyphenols". *Current Pharmaceutical Design* 19.34 (2013): 6186-6206.
14. Salganik RI. "The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population". *The Journal of the American College of Nutrition* 20.5 (2001): 464S-472S.
15. Basu A and Penugonda K. "Pomegranate juice: A heart healthy juice". *Nutrition Reviews* 67.1 (2009): 49-56.

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16. Sumner MD., *et al.* "Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease". *American Journal of Cardiology* 96.6 (2005): 810-814.
17. Laslett LJ, *et al.* "The Worldwide Environment of Cardiovascular Disease: Prevalence, Diagnosis, Therapy, and Policy Issues: A Report From the American College of Cardiology". *Journal American College of Cardiology* 60.25 (2012): 1-49.
18. Hollman PCH, *et al.* "The Biological Relevance of Direct Antioxidant Effects of Polyphenols for Cardiovascular Health in Humans is not established". *Journal of Nutrition* 141.5 (2011): 989S-1009S.
19. Decorde K., *et al.* "Chardonnay grape seed procyanidin extract supplementation prevents high-fat diet-induced obesity in hamsters by improving adipokine imbalance and oxidative stress markers". *Molecular Nutrition & Food Research* 53.5 (2009): 659-666.
20. Georgiev V., *et al.* "Recent advances and uses of grape flavonoids as Nutraceuticals". *Nutrients* 6 (2014): 391-415.
21. Prochazkova D., *et al.* "Antioxidant and prooxidant properties of flavonoids". *Fitoterapia* 82.4 (2011): 513-523.
22. Pietta PG. "Flavonoids as antioxidants". *Journal of Natural Products* 63.7 (2000): 1035-1042.
23. Singleton VL and Rossi JA. "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic reagents". *American Journal of Enology and Viticulture* 16.3 (1965): 144-158.
24. Buege JA and Aust SD. "Microsomal lipid peroxidation". *Methods in Enzymology* 52 (1978): 302-310.
25. Bland M. "Directory of randomisation software and services". (2004).
26. Krikorian R., *et al.* "Blueberry Supplementation Improves Memory in Older Adults". *Journal of Agricultural and Food Chemistry* 58.7 (2010): 3996-4000.
27. Gordillo G., *et al.* "Oral Administration of Blueberry Inhibits Angiogenic Tumor Growth and Enhances Survival of Mice with Endothelial Cell Neoplasm". *Antioxidants & Redox Signaling* 11.1 (2009): 47-58.
28. Zern TL., *et al.* "Grape Polyphenols Exert a Cardioprotective Effect in Pre- and Postmenopausal Women by Lowering Plasma Lipids and Reducing Oxidative Stress". *Journal of Nutrition* 135 (2005): 1911-1917.
29. Hichri I., *et al.* "Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway". *Journal of Experimental Botany* 62 (2011): 2465-2483.
30. O'Byrne DJ., *et al.* "Comparison of the antioxidant effects of Concord grape juice flavonoids α -tocopherol on markers of oxidative stress in healthy adults". *American Journal of Clinical Nutrition* 76.6 (2002): 1367-1374.
31. Marangon K., *et al.* "Comparison of the effect of alpha-lipoic acid and alpha-tocopherol supplementation on measures of oxidative stress". *Free Radical Biology & Medicine* 27.9-10 (1999): 1114-1121.
32. Morillas-Ruiz JM *et al.* "Effects of polyphenolic antioxidants on exercise-induced oxidative stress". *Clinical Nutrition* 25.3 (2005): 444-453.
33. Dillard CJ., *et al.* "Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation". *Journal of Applied Physiology* 87 (1978): 2032-2036.
34. Bejma J and Ji LL. "Aging and acute exercise enhance free radical generation in rat skeletal muscle". *The American Physiological society* 87.1 (1999): 465-470.
35. Jackson MJ and McArdle A. "Age-related changes in skeletal muscle reactive oxygen species generation and adaptive responses to reactive oxygen species". *The Journal of Physiology* 589.9 (2011): 2139-2145.
36. Aguilo A., *et al.* "Antioxidant response to oxidative stress induced by exhaustive exercise". *Physiology & Behavior* 84.1(2005): 1-7.
37. Garcia-Alonso J., *et al.* "Acute intake of phenolic-rich juices improves antioxidant status in healthy subjects". *Nutrition Research* 26.7 (2006): 330-339.
38. Spormann TM., *et al.* "Anthocyanin/polyphenolic-rich fruit juice reduces oxidative cell damage in an intervention study with patients on hemodialysis". *Cancer Epidemiology, Biomarkers & Prevention* 17.12 (2008): 3372-3380.
39. Costantini F., *et al.* "Effect of a standardized grape seed extract on low-density lipoprotein susceptibility to oxidation in heavy smokers". *Metabolism* 52.10 (2013): 1250-1257.

40. Srividhya R., *et al.* "Attenuation of senescence-induced oxidative exacerbations in aged rat brain by (-)-epigallocatechin-3-gallate". *International Journal of Developmental Neuroscience* 26.2 (2008): 217-223.
41. Roura E., *et al.* "Total polyphenol intake estimated by a modified folin-ciocalteu assay of urine". *American Association of clinical chemistry* 52 (2006): 749-752.
42. Benzie IF and Strain JJ. "The ferric reducing ability of plasma (FRAP) as a measure of 'Antioxidant power'. The FRAP assay". *Analytical Biochemistry* 239.1 (1996): 70-76.
43. Ghiseli A., *et al.* "Total antioxidant capacity as a tool to assess redox status: critical view and experimental data". *Free radical biology and medicine* 29.11 (2000): 1106-1114.
44. Schofield D and Braganza JM. "Shortcomings of an automated assay for total antioxidant status in biological fluids". *clinical chemistry* 42.10 (1996): 1712-1714.
45. Brown EM., *et al.* "Comparison of in vivo and in vitro digestion on polyphenol composition in lingonberries: potential impact on colonic health". *Biofactors* 40.6 (2014): 611-623.
46. Pimpão RC., *et al.* "Urinary metabolite profiling identifies novel colonic metabolites and conjugates of phenolics in healthy volunteers". *Molecular Nutrition & Food Research* 58.7 (2014): 1414-1425.

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