Original article

Effects of watermelon and Tualang honey based energy drinks on postprandial antioxidant activity and oxidative stress in male collegiate athletes: A dose-response and time-course efficacy study

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Abstract. *Background:* Exogenous antioxidant supplementation via natural food sources may enhance antioxidant activity and reduce oxidative stress. This study examined the dose-response and time-course effect of watermelon and Tualang honey based energy drinks (WED) on postprandial antioxidant activity and oxidative stress in male collegiate athletes. *Methods:* This randomized, two-dose, crossover design study included 12 healthy male collegiate athletes. Participants consumed WED of 0.5 g CHO/kg (WML) or 1g CHO kg (WMH) on two occasions separated by 7 days washout period. The primary outcome ferric reducing antioxidant power (FRAP) and secondary outcomes total phenolic content (TPC), reactive oxygen species (ROS) and malondialdehyde (MDA) were analyzed in blood samples drawn at baseline (fasting) and at 30, 60, 90, 120 minutes post-ingestion of WED's. *Results:* The area under the curve for FRAP (AUC_{FRAP}) and ROS (AUC_{ROS}) was higher ($p = 0.024$) and lower ($p = 0.021$) respectively in WMH compared to WML trial. AUC_{TPC} was higher ($p = 0.001$) in WML, whereas, AUC_{MDA} showed no significant difference ($p > 0.05$) between both trials. Concentrations of ROS and MDA in plasma significantly decreased ($p < 0.05$) from baseline to 60 min post-consumption of WMH. Plasma FRAP concentration peaked ($p > 0.05$) at 60 min post ingestion of WMH, whilst it showed constant decline post consumption of WML. Plasma TPC concentration peaked ($p < 0.05$) at 60 min in WML, whereas it increased ($p > 0.05$) over time in WMH trial. *Conclusions*: WMH demonstrated an optimal increment in antioxidant activity and a decrease in oxidative stress at 60 min after its ingestion among male collegiate athletes.

Key words: Antioxidants, Athletes, Dosage, Ergogenic aids, Oxidative stress

Introduction

Aerobic organisms persistently generate free radicals due to natural metabolic processes. These highly reactive chemical species which may be formed from oxygen molecules consist of free electrons in their outermost shell and are able to oxidize other compounds are referred to as reactive oxygen species (ROS) (1). However, exogenous factors, such as, pollution, smoking, ionizing radiation and psychophysical stress due to extensive physical activity may also contribute to production of free radicals and ROS (2). In general, free radicals are beneficial for immune function and cellular signaling, however, supraphysiological amounts

and/or deficiency of endogenous antioxidant system causes disproportionate formation and expulsion of ROS leading to oxidative damage of lipids, proteins and nucleic acids (3). In athletes, exhausting and vigorous training may result in excessive production of ROS, limiting capacity of endogenous antioxidants to counter abundant free radicals leading to oxidative stress that can cause muscle weakness and fatigue (4). Thus, exogenous antioxidant supplementation providing support to existing endogenous antioxidant system may be a valuable strategy to counter exercise-induced oxidative stress (5).

Pre-exercise antioxidant supplementation can inhibit production of ROS by scavenging the oxidants developed during biological metabolism or safeguarding proteins, lipids and nucleic acids against any disturbance by free radicals (6). Fruits are considered rich source of antioxidants. Hence, their supplementation might act as a good nutritional strategy to improve antioxidant activity while countering oxidative stress, resulting in enhanced exercise performance (7). Fruits such as berries (8), cherries (9), pomegranate (10) and grapes (11) have been shown to increase plasma antioxidant activity and lower oxidative stress response in both non-athletes and athletes. Supplementation of antioxidant rich concentrate of berries (500 mL of 5% concentrate) demonstrated increased ($p < 0.05$) postprandial antioxidant capacity (2,2-diphenyl-1-picrylhydrazyl) along with decreased ($p < 0.05$) malondialdehyde plasma concentration in healthy males (8). Another study reported 43.6% increase in total antioxidant capacity after the consumption of 10 mL/kg of whole grape juice 2 hours before a run to exhaustion in recreational male runners (12).

Meanwhile, a fruit rich in antioxidants (13) and whose absence in the field of sports nutrition research is felt is watermelon. Watermelon (*Citrullus lanatus*) belongs to *Cucurbitaceae* plant family and is extensively cultivated in Malaysia (14). It is a rich source of nutrients such as vitamins (B, C and E), minerals (phosphorus, magnesium, calcium and iron), amino acids and phytochemicals such as carotenoids (lycopene and β-carotene) and polyphenols (15). Watermelon constitutes approximately 90% water and contains 6-8 g CHO per 100 g of fruit (16). Besides, it is also a rich source of electrolytes such as potassium which helps in regulating fluid balance, muscle contraction and nerve signals (17). Therefore, watermelon juice is being consumed as an alternative pre and post-workout drink by active individuals and athletes. Past studies have mainly focused on the effects of acute or long term supplementation of watermelon on physiological parameters such as cycling time-trial (18), time to exhaustion (19), maximum effort test (20) and fatigue index (21). In animal studies, watermelon has been shown to enhance antioxidant activity and reduce oxidative stress after its consumption (22,23). However, similar studies on humans are very limited. Furthermore, the effects of dosage and timing of watermelon intake on these parameters have not been studied, particularly in male athletes. Energy drinks is not only a source of carbohydrate to improve exercise performance, but can also be a source of natural antioxidants that can protect against exercise-induced oxidative damage. It has been suggested that a mixture of bioactive compounds is predicted to show better protection against oxidative stress compared to a single compound (24). Thus, in the present study, Tualang honey was added to the WED due to its high antioxidant activities and to meet the 0.5g carbohydrate (CHO)/kg and 1g CHO/kg body weight of dosages based on pre-exercise carbohydrate recommendation of 30-60g or 1g CHO/kg body weight, 1-2 hour prior to exercise (25,26).

This study aimed to investigate the dose-response effect of WED on postprandial antioxidant activity and oxidative stress biomarkers and to determine the time-course effect that could provide optimal protection against oxidative damage in male collegiate athletes. The findings will aid in providing scientific evidence of the optimum dosage and timing of pre-exercise WED consumption, which may further help in revising pre-exercise nutritional recommendations for athletes.

Methods

Study design and ethics

This study employed randomized, crossover design. Randomization was conducted by an individual not involved in data collection using an online program (randomizer.org). In a simple 1:1 randomization

approach, participants consumed 0.5g CHO/kg body weight (WML) in the first visit and 1g CHO/kg body weight (WMH) of WED in the second visit or vice-versa after an overnight fast on two different occasions separated by a one-week washout period. During the 3 days leading up to the first experimental trial, the participants recorded their dietary intake and physical activity and were asked to replicate this before their second trial. Additionally, they were instructed limit themselves to activity of daily living and not to consume foods high in phytochemicals, alcohol and sports supplements. For 24 h before each experimental trial, the participants were asked to strictly refrain from exercise. Both experimental trials were conducted during the same time slot for each participant. During the trials participants were asked to remain seated inside the testing premises accomplishing sedate behavior.

This study was conducted at sports nutrition laboratory, Faculty of Sports and Exercise Science, Universiti Malaya after receiving approval from the Universiti Malaya Research Ethics Committee (Protocol no: UM.TNC2/UMREC-331). All procedures performed in this study involving human participants were in agreement with the declaration of Helsinki 1964 and all participants were administered with written informed consent prior to their participation in the study. There were no amendments in the experimental protocols after the commencement of the study.

Participants

Twelve healthy male collegiate athletes were recruited from among residential students of Universiti Malaya via recruitment flyers. The sample size was determined using G*Power software (version 3.1.9.2). Ferric reducing antioxidant power (FRAP) was the primary outcome and thus used as the main variable for sample size calculation based on the results of past study (28). The calculation determined a minimum sample size of 10 subjects with effect size = 0.45, $\alpha = 0.05$ and statistical power 0.8. However, to allow for potential withdrawals the final sample size was increased to 12 subjects. Women were excluded to avert the assessment of different hormonal states. The participants were selected according to the following inclusion criteria: (1) aged 18-25 years (2) BMI within healthy range (18.5-25.0 kg/m^2) (3) have been participating in sports competitions at university level for at least 3 years (4) not suffering from any illness or chronic diseases (5) not taking any medication (6) not a consumer of dietary and sports supplement. Participants were excluded if they reported any cardiovascular, metabolic or digestive ailment, food allergies and smokers.

Preparation of watermelon and Tualang honey based energy drinks

Nutritional compositions were estimated using Nutritionist Pro^{TM} software version 5.3.0 (Axxya Systems LLC, Redmond, USA). WML and WMH were prepared to serve 0.5g CHO/kg and 1g CHO/kg body weight respectively, i.e., each drink served the required CHO content corresponding to participant's body weight. Both variation of WED's comprised of fresh watermelon and Tualang honey in 1:1 ratio of required CHO content. Hence, the amount of watermelon and Tualang honey separately used were 0.25g CHO/kg in WML and 0.5g CHO/kg in WMH test drinks. Watermelon cubes were weighed on a digital kitchen weighing scale (ENDO, Japan, E-DKS3650) and their extract was obtained using a blender (Philips HR1363/04). The extract was filtered using fine muslin cloth. Tualang honey was weighed to serve the remaining half of required carbohydrate content and added to filtered watermelon juice. Lastly, 5g (WML) and 10g (WMH) of calamondin lime was added to elevate the taste of drinks. The drinks were prepared fresh in the morning before each experimental trial and refrigerated at 5 °C until served in an identical sipper bottles.

Experimental trial

Participants were asked to sit for ten minutes upon their arrival at sports nutrition laboratory, Faculty of Sports and Exercise Science, Universiti Malaya. Subsequently, their body composition (Inbody 370, USA) and blood pressure (Omron HEM7121, Japan) were measured. A butterfly catheter (BD, Japan) was inserted in an antecubital vein for repeated blood collection. After the withdrawal of baseline fasting blood sample, an assigned WED was consumed by the

participants within 5 min. Participants were blinded to the test beverages. Blood was withdrawn (6 mL) at 30, 60, 90 and 120 min post-consumption of the experimental beverages. The collected blood samples were centrifuged (Tomi CAX-371, Japan) at 3000 rev/min for 15 minutes at 4°C. Subsequently, aliquots of plasma samples were pipetted into 1.5 mL micro centrifuge tubes, and stored in the refrigerator at - 40°C before analyzed for, ferric reducing antioxidant activity (FRAP), total phenolic content (TPC), reactive oxygen species (ROS) and malondialdehyde (MDA).

Analysis of antioxidant activity, total phenolic content and oxidative stress biomarkers

Ferric reducing antioxidant power (FRAP)

Plasma FRAP concentration was determined using the method of Benzie and Strain (27) by which, 300 mM acetate buffer, 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 20 mM ferric chloride solution ($FeCl_3$) and 40 mM hydrochloric acid (HCL) were prepared for the assay. Working reagent was made by mixing 10 mL of acetate buffer along with 1 mL TPTZ solution and 1 mL $FeCl_3$ solution. In a standard 96-well microtiter plate, 5 µL of plasma sample was loaded with 300 µL of freshly prepared working reagent and the mixture was incubated at room temperature for 30 min. The absorbance was read at 593 nm (Bio-Plex® 200: Bio-Rad, USA). A standard curve was plotted using Iron sulphate (0-1000 µM) as standard and the results were expressed as µM of ferrous ion (Fe^{2+}). All analysis was performed in triplicates.

Total phenolic content (TPC)

TPC was estimated using Folin-Cocalteu assay (28) by which, 10 µL of plasma was added with 500 µL of 1N Folin-Cocalteu reagent (FCR) in a standard 96-well microtiter plate and incubated for 5 min at room temperature. Subsequently, 350 µL Na_2Co_3 was added and vortexed for 10s. The mixture was wrapped in aluminum foil and incubated for 2 hour at ambient temperature. Absorbance reading was recorded at 765 nm (Bio-Plex® 200: Bio-Rad, USA). A standard curve was plotted using Gallic acid (0-400 µg/mL). The results were expressed as µg of Gallic acid equivalents per mL of plasma (GAE). All analysis was performed in triplicates.

Reactive oxygen species (ROS)

ROS was analyzed using the method described in Kong et al. (29) with slight modification, in which dichlorofluorescein diacetate (DCFH-DA) was adopted as the fluorescence-based probe for identifying ROS in plasma. In a 96-well black microplate, 5 µL of plasma sample was mixed with 100 µL of dichlorofluorescein diacetate reagent (20 µM) and incubated for 30 min at ambient temperature. Subsequently, fluorescence readings were recorded at 485 (excitation) and 530 (emission) nm using a multiplex array system (Bio-Plex® 200: Bio-Rad, USA). All results were expressed as relative fluorescence unit (U).

Malondialdehyde (MDA)

Lipid peroxidation in the plasma samples were estimated using thiobarbituric acid reactive substances (TBARS) assay. MDA reacts with thiobarbituric acid (TBA) to produce TBARS (30). TBARS reagent was prepared fresh and composed of 12 g trichloroacetic acid (TCA), 0.3 g of TBA and 1.04 mL of 70% perchloric acid ($HCLO_4$) mixed in 80 mL of double distilled water. For the assay, 50 µL of plasma sample was added with 250 µL of TBARS reagent in a micro centrifuge tubes and heated at 90 °C for 20 min. Thereafter, tubes containing the mixture were snap chilled and centrifuged at 900 rcf (relative centrifugal force) for 10 min. Subsequently, 50 µL supernatant was pipetted to 96-well standard microplate and absorbance was recorded at 532 nm using a spectrophotometer (Bio-Plex® 200: Bio-Rad, USA). A standard calibration curve was plotted using 1,1,2,2 - tetraethoxypropane (0-100ng/µL) as standard. All analyses were performed in triplicates.

Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). The significance level was set

at $p < 0.05$. The baseline and body composition data of the participants are presented as means ± SD. The area under the curve (AUC) was calculated using trapezoidal formula and the differences between WML and WMH were analyzed using paired sample t-test. Shapiro–Wilk tests were used to examine if the data were normally distributed. Repeated measures analysis of variance (ANOVA) was performed, determining the effect of treatment, time and interaction of treatment × time. Mauchley's test of sphericity was used to report within-subject (time) effects of equal variance. However, Greenhouse Geisser corrections were reported where assumption of equal variance were violated. Post hoc analyses were performed if there were significant interactions. A one-way repeated measures ANOVA was performed to analyze any changes between time-points.

Results

All twelve participants completed both experimental trials with no dropout during the course of experiments and the baseline physical characteristics are presented in Table 1. The recruitment and enrolment data are presented in Figure 1. No adverse events were reported during the study and the participants adhered to all the instructions provided prior to the start of study.

Area under the curve

The AUC_s for plasma FRAP, TPC, ROS and MDA over two hours post ingestion of WML and WMH are shown in Table 2. The AUC for FRAP in WMH trial was significantly ($p < 0.05$) higher and for ROS and TPC were significantly lower as compared to WML trial. No significant difference was found between AUC for MDA.

Table 1. Physical characteristics of participants at baseline.

Variables	n = 12
Age (year)	20.5 ± 0.9
Weight (kg)	66.37 ± 7.89
Height (cm)	171.75 ± 5.57
BMI (kg/m^2)	22.47± 2.16
FFM (kg)	54.32 ± 6.49
BF (%)	17.91 ± 5.81

Data expressed as Mean ± SD. BMI, Body mass index; FFM, Free fat mass; BF, Body fat.

Postprandial plasma FRAP, TPC, ROS and MDA concentrations over time following the consumption of watermelon and Tualang honey based energy drinks

Ferric reducing antioxidant power (FRAP)

FRAP concentration at fasting and at 30, 60, 90, 120 min following WML and WMH consumption are presented in Table 3. Plasma FRAP concentrations showed significant main effect $F(1,11) = 5.99$, $p < 0.05$, $\eta_p^2 = 0.35$ between WML and WMH trials with a significant main effect, $F(2.19,24.11) = 6.34$, $p < 0.05$, $\eta_p^2 = 0.36$ of dose*time. However, no significant main effect of time-points ($p > 0.05$) was observed. Maximum FRAP activity was reported after 60 min post ingestion of WMH (Table 3), showing an increment of 4.39% from baseline which declined towards baseline by 120 min (Figure 3a). Meanwhile, no increment in FRAP activity was observed post ingestion of WML throughout the experiment lasting 120 min (Figure 3a). No significant changes were perceived at all time-points for both WML and WMH trials.

Total phenolic content (TPC)

TPC concentration at fasting and at 30, 60, 90, 120 min following WML and WMH consumption are presented in Table 3. Plasma TPC concentrations showed significant main effect $F(1,11) = 257.13$, $p < 0.05$, $\eta_p^2 = 0.96$ between WML and WMH trials with a significant main effect $F(4,44) = 2.93$, $p < 0.05$, $\eta_p^2 = 0.21$ of time-points. However, no significant main effect of dose*time ($p > 0.05$) was observed. Plasma TPC concentration increased ($p > 0.05$) from baseline to 60 min and remained constant until 120 min post ingestion of WMH (Table 3) representing an increment of 3.85 % compared to baseline (Figure 3b). Whilst in WML, plasma TPC levels showed significant increased ($p < 0.05$) and reached

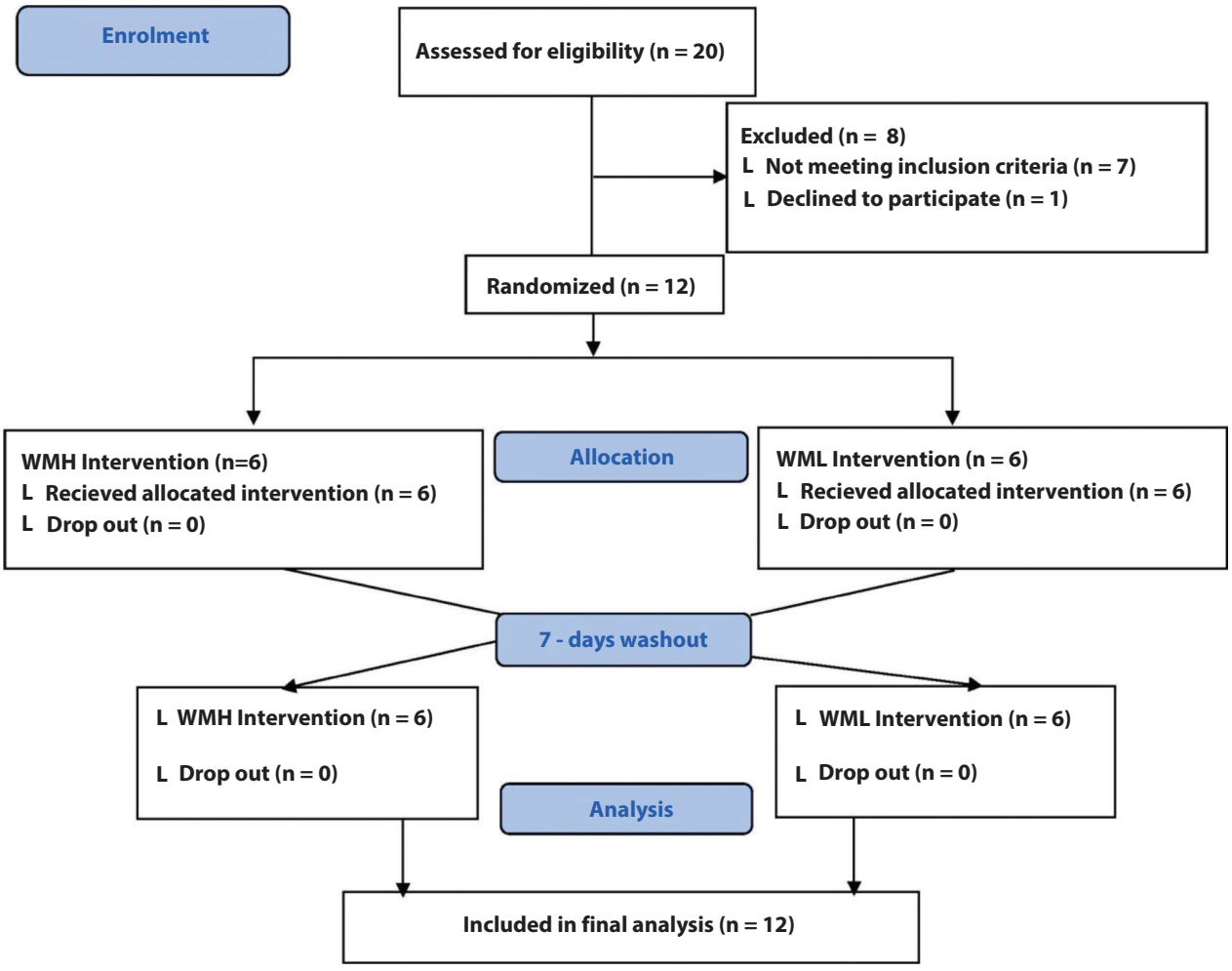

Figure 1. Flow diagram of recruitment and enrolment of participants.

Table 2. Area under the curve for antioxidant capacity, total phenolic content and biomarkers of oxidative stress following consumption of watermelon-based energy drinks.

Markers	WML (0.5g/kg)	WMH (1g/kg)	*p*
FRAP (µM)	1368.488 ± 85.863	1660.486 ± 88.98*	0.024
TPC (GAE µg/mL)	2938.645 ± 31.03*	2443.487 ± 23.64	0.001
ROS (U)	127.563 ± 2.744*	120.869 ± 3.593	0.021
MDA (ng/µL)	84.416 ± 1.49	85.955 ± 1.115	0.412

Data indicated as Mean ± SEM; n = 12; * Significant difference ($p < 0.05$) in values using paired t-test.
SEM, Standard error of the mean; WML, Watermelon low dose; WMH, Watermelon high dose; FRAP, Ferric reducing antioxidant power; TPC, Total phenolic content; ROS, Reactive oxygen species; MDA, Malondialdehyde

maximum level at 60 min measuring 4.48 % increase compared to baseline, after which the levels declined toward baseline by 120 min post ingestion (Table 3, Figure 3b).

Reactive oxygen species (ROS)

Plasma ROS concentration following consumption of both doses of WED are presented in

Table 3. Biomarkers of antioxidant activity, phenolic content and oxidative stress at baseline (fasting) and at 30,60,90,120 min post ingestion of watermelon-based energy drinks.

Markers	Time-points				
	Baseline	**30 min**	**60 min**	**90 min**	**120 min**
FRAP (μM)					
WML	796.290 ± 35.867	686.112 ± 56.136	666.410 ± 52.424	656.706 ± 44.015	659.188 ± 42.799
WMH	809.048 ± 47.693	843.491 ± 50.747	844.583 ± 43.850	819.226 ± 40.344	818.270 ± 44.863
TPC (GAE μg/mL)					
WML	1410.625 ± 26.737	1475.625 ± 22.190	1499.791 ± 25.234	1465.208 ± 15.086	1437.708 ± 14.778
WMH	1187.291 ± 18.908	1221.875 ± 19.666	1227.708 ± 14.966	1229.163 ± 19.572	1229.164 ± 26.759
ROS (U)					
WML	63.830 ± 1.824	62.796 ± 1.671	62.546 ± 1.535	65.190 ± 1.567	65.358 ± 1.573
WMH	63.009 ± 1.711	60.038 ± 1.742*	59.728 ± 1.805*	60.085 ± 1.855	60.765 ± 2.075
MDA (ng/μL)					
WML	43.186 ± 0.985	41.927 ± 0.937	41.767 ± 0.690	42.228 ± 0.845	42.591 ± 1.084
WMH	44.276 ± 0.937	42.486 ± 0.818	41.523 ± 0.816*	43.328 ± 0.993	44.507 ± 1.222

Data expressed as Mean + SEM; n = 12; *Values significantly (p<0.05) different from baseline using one-way repeated measures ANOVA.
SEM, Standard error of the mean; ANOVA, Analysis of variance; WML, Watermelon low dose (0.5g/kg) ; WMH, Watermelon high dose (1g/kg); FRAP, Ferric reducing antioxidant power; TPC, Total phenolic content; ROS, Reactive oxygen species; MDA, Malondialdehyde

Table 3. Plasma ROS concentrations over 2 hours post-consumptions of WED revealed a significant main effect $F(1,11) = 5.74$, $p < 0.05$, $\eta_p^2 = 0.34$ between WML and WMH trials. However, no significant main effect ($p > 0.05$) of time-points and dose*time was observed. Plasma ROS concentration showed a reduction of 5.25 % ($p < 0.05$) at 60 min time-point in the WMH trial and 1.82 % ($p > 0.05$) in the WML trial compared to their respective baseline (Table 3, Figure 3c) .

Malondialdehyde (MDA)

There was no significant ($p > 0.05$) main effect of dose, time-points and dose*time on postprandial plasma MDA levels following ingestion of WML and WMH (Table 3). Plasma MDA concentration reached maximum reduction of 3.07% and 6.05% at 60 min from baseline for both WML and WMH trials, respectively (Figure 3d).

Discussion

As shown in Figure 2, the main findings demonstrated that WMH (1g CHO/kg) dosage of WED increased antioxidant activity while exhibiting protective nature against oxidative damage when compared to WML. Furthermore, time-course efficacy of WMH for optimal antioxidant activity and protection against oxidative stress was 30 – 90 min after its ingestion. To the best of our knowledge, this is the first study examining the dose-response and time-course efficacy effects of exogenous antioxidant supplementation through watermelon and Tualang honey based energy drinks on postprandial antioxidant activity and oxidative stress response among male collegiate athletes.

Recent nutritional studies have concentrated on supplementation via natural foods constituting high concentration of phenolic compounds possessing antioxidant and anti-inflammatory properties that may avert exercise-induced muscle damage, attenuate inflammation and oxidative stress as well as help the athletes to recover from the oxidative stress produced by free radicals (31). Howatson et al. (32) in a recent study reported that acute supplementation with haskap berries rich in anthocyanins resulted in enhanced VO_2 max and delayed time to exhaustion in male recreational runners. Another study reported increased total antioxidant capacity (TAC) and superoxidase dismutase (SOD) activities, and mitigated malondialdehyde (MDA) levels during the recovery period ($p < 0.05$) after supplementation of quercetin for 7 days among healthy male participants (33). The

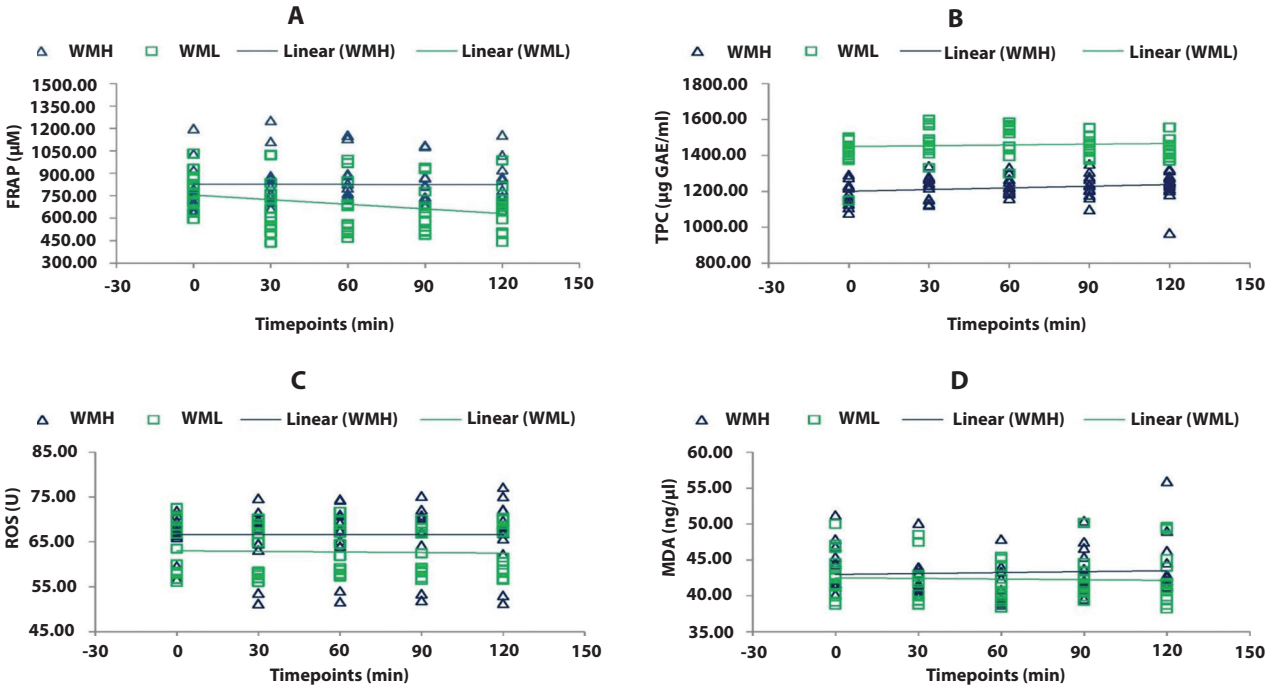

Figure 2. Mean plasma concentration-timepoint profiles following ingestion of 0.5g CHO/kg (WML) and 1g CHO/kg (WMH) dosages of watermelon and Tualang honey based energy drinks (WED's) A) Ferric reducing antioxidant power (FRAP) B) Total phenolic content (TPC) C) Reactive oxygen species (ROS) D) Malondialdehyde (MDA).

study further reported significant improvement in 75% $\dot{V}O_{2max}$ cycling performance after quercetin treatment and accompanied by lower responses of interleukin 6 and Creatine Kinase.

In the current study, the increase in postprandial antioxidant activity post ingestion of WMH exhibit potential protective effect against oxidative damage, which was further supported by decreased concentration of MDA and ROS, is signifying reduced levels of oxidative stress. This finding is in agreement with a previous study (22), which reported enhanced antioxidant activity as determined by increased FRAP and oxygen radical absorbance capacity (ORAC) after consuming watermelon puree for 2 weeks. Data on human trials is limited; however, earlier studies conducted on experimental animals have also reported similar effects of increased antioxidant activity with lower oxidative stress post-consumption of watermelon (23). This increase in antioxidant activity can be attributed to the nutrient content of watermelon, in particular lycopene (34) and other carotenoid antioxidants (35). In addition, L-citrulline which is a naturally occurring amino acid may acts as a substrate for endogenous nitric oxide (NO) production post-consumption of watermelon (36). NO, mainly known for its vasodilatory abilities, can help in reducing oxidative stress by scavenging or inhibiting the production of hydroxyl radicals. Furthermore, Tualang honey contains high amount of phenolic acids (37), high free radical scavenging (38) and antioxidant activity (39) which has been shown to protect against oxidative stress after its consumption in a postprandial study conducted by Ahmed et al. (40).

Optimum increase in antioxidant activity was seen in the first 60 min post-consumption of WMH compared to WML condition which saw constant decrease in antioxidant activity. This elevation in antioxidant activity might be due to prompt bioavailability and higher antioxidants and phenolic compounds in the blood post ingestion of WMH. A similar trend of increasing antioxidant activity within the first hour was seen in the previous study post-consumption of Tualang honey (40). However; the decline in antioxidant activity post WML ingestion may indicate the inability of the lower dose to counter ROS and free radicals damages. Plasma

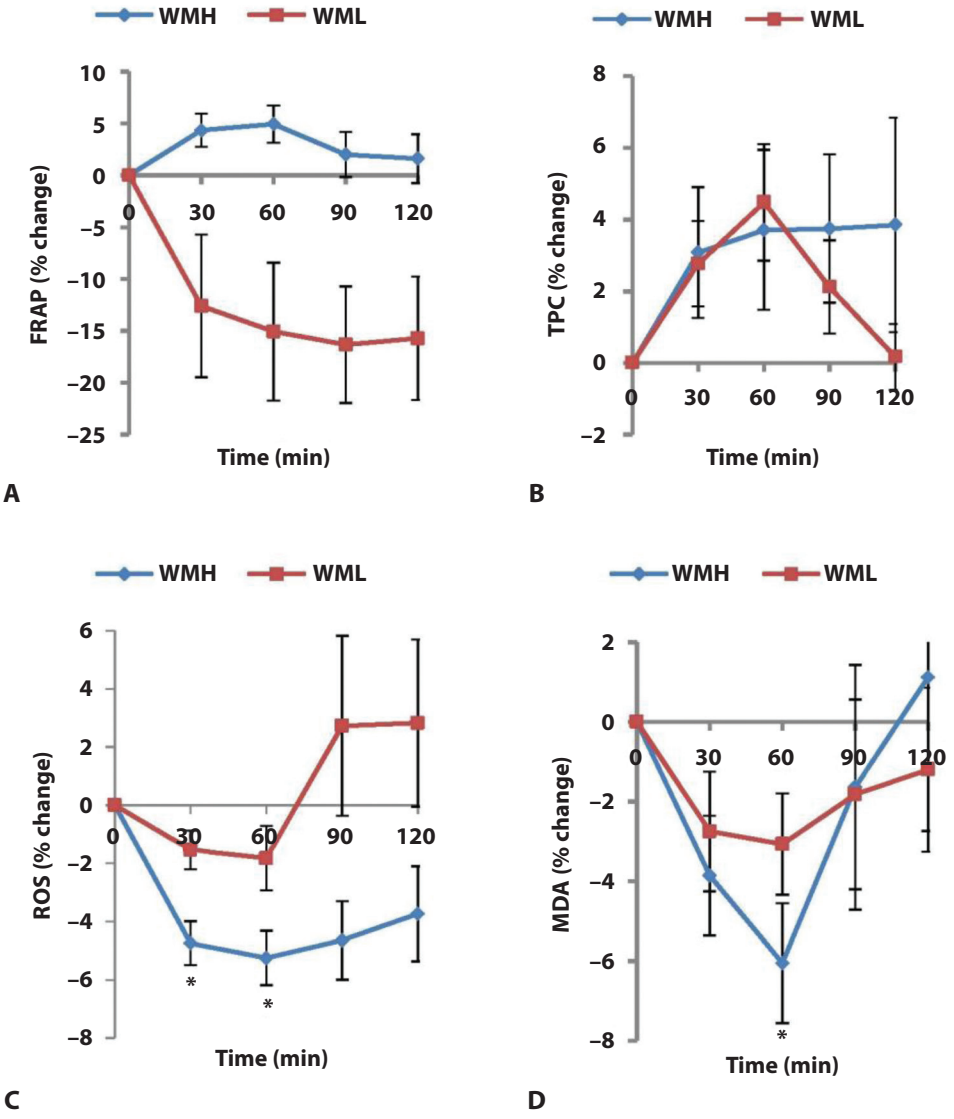

Figure 3. Percentage change of plasma ferric reducing antioxidant power (FRAP) (a) total phenolic content (TPC) (b) reactive oxygen species (ROS) (c) and malondialdehyde (MDA) (d) before baseline and at 30,60,90,120 min after watermelon-based energy drinks consumption in WML (0.5g/kg) and WMH (1g/kg). Data expressed as Mean + SEM; n = 12; *Values significantly (p<0.05) different from baseline.

TPC concentration showed optimum increment at 60 min post-consumption of WML and constantly rose until 120 min in WMH. The higher concentration of plasma TPC may have resulted from the rapid absorption of polyphenols by passive diffusion (41) which is seen in case of juices or beverages compared to complex foods having low absorption rate (42).

Oxidative stress may impair cellular units including lipids. MDA is formed due to overproduction of ROS or free radicals, demonstrating the elevated pace of lipid peroxidation (43). The effects of WED against lipid peroxidation were investigated and the findings revealed that postprandial MDA concentration decreased to optimum level at 60 min post-consumption of both doses. This was concluded by the similar trend observed in the postprandial ROS concentration and hence demonstrated the capability of WED to counter ROS induced lipid peroxidation.

The use of crossover design in this study contributes to minimum confounders; however, there are several limitations that must be taken into account while drawing evidence based interpretation. This study did not estimate the antioxidant and phenolic contents of watermelon and Tualang honey. Also, the activity of antioxidant enzymes was not examined as it aimed to focus on exogenous antioxidant supplementation via watermelon and Tualang honey based energy drinks. Furthermore, oxidative damage to nucleic acids or proteins was not assessed, inhibiting outcomes of lipid peroxidation. The recruitment of only male participants might limit the universality of the findings of this study. Future studies may include an extended arm(s) of honey and/or watermelon separately should focus on analyzing the chronic effects of WED consumption involving diverse population groups. Furthermore, in order to determine the optimal concentration, intake timing and dosage, more trials' assessing the effects of WED's in larger sample size is warranted.

Conclusion

This study concludes that 1g/kg (WMH) dosage of WED demonstrated increase in antioxidant activity complemented with decrease in oxidative stress biomarkers in male collegiate athletes exhibiting protective nature against oxidative damage. The time-course efficacy of WMH for optimum antioxidant activity and protection against oxidative stress was 60 min after its consumption. This study highlights the usage of watermelon based energy drink as natural and effective source of exogenous antioxidant supplementation. Assessing the effect of pre-exercise consumption of WED on exercise performance may ultimately aid in developing a nutritional ergogenic aid and pre exercise fueling recommendations.

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Competing Interests: The authors declare that there is no conflict of interest.

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References

1. Nocella C, Cammisotto V, Pigozzi F, et al. Impairment between oxidant and antioxidant systems: Short and long-term implications for athletes' health. Nutrients 2019; 11(6): 1353.
2. Kawamura T, Muraoka I. Exercise-induced oxidative stress and the effects of antioxidant intake from a physiological viewpoint. Antioxidants 2018; 7: 1-19.
3. Goulart MJVC, Pisamiglio DS, MÖller GB, et al. Effects of grape juice consumption on muscle fatigue and oxidative stress in judo athletes: a randomized clinical trial. An Acad Bras Cienc 2020; 92(4): e20191551.
4. Thirupathi A, Pinho RA. Effects of reactive oxygen species and interplay of antioxidants during physical exercise in skeletal muscles. J Physiol Biochem 2018; 74(3): 359–367.
5. Sellami M, Slimeni O, Pokrywka A, et al. Herbal medicine for sports: a review. J Int Soc Sports Nutr 2018; 15: 14.
6. Matschke V, Theiss C, Matschke J. Oxidative stress: the lowest common denominator of multiple diseases. Neural Regen Res 2019; 14(2): 238–241.
7. Bowtell J, Kelly V. Fruit-derived polyphenol supplementation for athlete recovery and performance. Sports Med 2019; 49(Suppl 1): 3–23.
8. Urquiaga I, Ávila F, Echeverria G, et al. A chilean berry concentrate protects against postprandial oxidative stress and increases plasma antioxidant activity in healthy humans. Oxid Med Cell Longev 2017; 8361493.
9. McCormick R, Peeling P, Binnie M, et al. Effect of tart cherry juice on recovery and next day performance in well-trained Water Polo players. J Int Soc Sports Nutr 2016;13(41):22.
10. Ammar A, Turki M, Chtourou H, et al. Pomegranate supplementation accelerates recovery of muscle damage and soreness and inflammatory markers after a weightlifting training session. PLoS ONE 2016; 11(10): e0160305.
11. Toscano LT, Tavares RL, Toscano LT, et al. Potential ergogenic activity of grape juice in runners. Appl Physiol Nutr Metab 2015; 40(9): 899–906.
12. de Lima Tavares Toscano L, Silva AS, de França A, et al. A single dose of purple grape juice improves physical performance and antioxidant activity in runners: a randomized, crossover, double-blind, placebo study. Eur J Nutr 2020; 59(7): 2997–3007.
13. Lum T, Connolly M, Marx A, et al. Effects of fresh watermelon consumption on the acute satiety response and cardiometabolic risk factors in overweight and obese adults. Nutrients 2019;11(3):595.
14. Ridwan R, Abduk Razak HR, Adenan MI, et al. Separation of L-arginine and L-citrulline in red and yellow crimson watermelon (Citrullus lanatus) juices extract using HPLC gradient mode. Malaysian J.Anal Sci 2018; 22(5): 785-793.

15. Jayaprakasha GK, Patil BS. A metabolomics approach to identify and quantify the phytochemicals in watermelons by quantitative 1 HNMR. Talanta 2016; 153: 268-277.
16. Maoto MM, Beswa D, Jideani AIO. Watermelon as a potential fruit snack. Int J Food Prop 2019; 22(1): 355-370.
17. Olayinka BU, Etejere EO. Proximate and chemical compositions of watermelon (Citrullus lanatus (Thunb.) matsum and nakai cv red and cucumber (Cucumis sativus L. ev Pipino). Int Food Res J 2018; 25(3): 1060-1066.
18. Shanely RA, Nieman DC, Perkins-Veazie P, et al. Comparison of watermelon and carbohydrate beverage on exercise-induced alterations in systemic inflammation, immune dysfunction, and plasma antioxidant capacity. Nutrients 2016; 8(8): 518.
19. Cutrufello PT, Gadomski SJ, Zavorsky GS. The effect of l-citrulline and watermelon juice supplementation on anaerobic and aerobic exercise performance. J Sports Sci 2015; 33(14): 1459-1466.
20. Tarazona-Diaz MP, Alacid F, Carrasco M, et al. Watermelon juice: potential functional drink for sore muscle relief in athletes. J Agric Food Chem 2013; 61(31): 7522-7528.
21. Rizal M, Segalita C, Mahmudiono T. The effect of watermelon beverage ingestion on fatigue index in young-male, recreational football players. Asian J Sports Med 2019; 10(2): e86555.
22. Hong MY, Hartig N, Kaufman K, et al. Watermelon consumption improves inflammation and antioxidant capacity in rats fed an atherogenic diet. Nutr Res 2015; 35(3): 251-258.
23. Hong MY, Beidler J, Hooshmand S, et al. Watermelon and l-arginine consumption improve serum lipid profile and reduce inflammation and oxidative stress by altering gene expression in rats fed an atherogenic diet. Nutr Res 2018; 58: 46-54.
24. Wei QY, Zhou B, Cai YJ, et al. Synergistic effect of green tea polyphenols with trolox on free radical-induced oxidative DNA damage. Food Chem 2006; 96(1): 90-95.
25. Kerksick CM, Arent S, Schoenfeld BJ, et al. International society of sports nutrition position stand: nutrient timing. J Int Soc Sports Nutr 2017; 14: 33.
26. Kerksick CM, Wilborn CD, Roberts MD, et al. ISSN exercise & sports nutrition review update: research & recommendations. J Int Soc Sports Nutr 2018; 15(1): 38.
27. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239(1): 70–76.
28. Cao G, Alessio HM, Cutler RG. Oxygen-radical absorbance capacity assay for antioxidants. Free Rad Biol Med 1993; 14: 303-311.
29. Kong KW, Mat-Junit S, Aminudin N, et al. Protective effects of the extracts of Barringtonia racemosa shoots against oxidative damage in HepG2 cells. PeerJ 2016; 4: 1–20.
30. Ramli NSF, Mat-Junit S, Leong NK, et al. Analyses of antioxidant status and nucleotide alterations in genes encoding antioxidant enzymes in patients with benign and malignant thyroid disorders. PeerJ 2017; 5: 3365.
31. Ortega DR, López AM, Amaya HM, et al. Tart cherry and pomegranate supplementations enhance recovery from exercise-induced muscle damage: a systematic review. Biol Sport 2021; 38(1): 97-111.
32. Howatson G, Snaith GC, Kimble R, et al. Improved endurance running performance following haskap berry (Lonicera caerulea L.) ingestion. Nutrients 2022; 14(4): 780.
33. Tsao JP, Bernard JR, Hsu HC, et al. Short-Term Oral Quercetin Supplementation Improves Post-exercise Insulin Sensitivity, Antioxidant Capacity and Enhances Subsequent Cycling Time to Exhaustion in Healthy Adults: A Pilot Study. Front Nutr 2022; 9: 875319.
34. Kim C-H, Park M-K, Kim S-K, et al. Antioxidant capacity and anti-inflammatory activity of lycopene in watermelon. Int J Food Sci Technol 2014; 49(9): 2083-2091.
35. Edwards AJ, Vinyard BT, Wiley ER, et al. Consumption of watermelon juice increases plasma concentrations of lycopene and beta-carotene in humans. J Nutr 2003; 133(4): 1043-1050.
36. Figueroa A, Wong A, Jaime SJ, et al. Influence of L-citrulline and watermelon supplementation on vascular function and exercise performance. Curr Opin Clin Nutr Metab Care 2017; 20(1): 92-98.
37. Mohd Kamal DA, Ibrahim SF, Kamal H, et al. Physicochemical and medicinal properties of Tualang, Gelam and Kelulut honeys: a comprehensive review. Nutrients 2021; 13(1): 197.
38. Kishore RK, Halim AS, Syazana MN, et al. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutr Res 2011; 31: 322–325.
39. Yaacob NS, Nengsih A, Norazmi MN. Tualang honey promotes apoptotic cell death induced by tamoxifen in breast cancer cell lines. Evid-based Complement Altern Med 2013; 989841.
40. Ahmad NS, Abdul AA, Kong KW, et al. Dose-Response effect of tualang honey on postprandial antioxidant activity and oxidative stress in female athletes: A pilot study. J Altern Complement Med 2017; 23: 989-995.
41. Ho KKHY, Ferruzzi MG, Wightman JD. Potential health benefits of (poly)phenols derived from fruit and 100% fruit juice. Nutr Rev 2020; 78(2): 145-174.
42. Swallah MS, Fu H, Sun H, et al. The impact of polyphenol on general nutrient metabolism in the monogastric gastrointestinal tract. J Food Qual 2020: 1-12.
43. Chusak C, Pasukamonset P, Chantarasinlapin P, et al. Postprandial glycemia, insulinemia, and antioxidant status in healthy subjects after ingestion of bread made from anthocyanin-rich riceberry rice. Nutrients 2020; 12(3): 782.

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