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ABSTRACT

This study aim is to determine the effects of short-term polysulfide-enriched garlic (PEG) on resting blood pressure (BP) and physiological responses to continuous, high-intensity exercise in collegiate-level athletes. Twelve collegiate-level male athletes underwent a randomized, double blind, crossover, placebo-controlled trial of PEG and PLA (placebo) supplementation, with a washout period of 14-day separating each trial. Following a 4-day supplementation of 4 g PEG and PLA (placebo), participants consumed a single dose of the supplement 3 hours prior to the completion of high-intensity exercise tolerance in Day-5. The systolic BP and mean arterial pressure levels were significantly lower ($p < 0.05$), following 5-day PEG supplementation, compared to the pre-supplementation (PRE; no supplement) and PLA condition, but not diastolic BP. In addition, exhaled hydrogen sulphide (eH_2S) was significantly greater ($p < 0.05$) following PEG, compared to PRE and PLA condition. Although PEG did not significantly alter time-to-exhaustion in intense constant load exercise ($p = 0.11$), the results indicate substantial improvements (~6%) in 8 out of 12 participants. Blood [glucose] was lower during constant-load exercise ($p < 0.05$) but no changes in blood [lactate]. In this study, for the first time the BP-lowering effect of PEG supplementation was reported, and this vasorelaxant effects likely related to enhanced bioavailability of hydrogen sulphide (H_2S). Additionally, there was appreciable inter-subject variability in the response of PEG on exercise tolerance which requires further study to elucidate the factors that influence its ergogenic potential.

Keywords: Garlic, Supplementation, Hydrogen Sulphide, Exercise Capacity, Blood Pressure

INTRODUCTION

The bio-compound from plants is widely researched for their health-promoting properties and disease prevention (Girish, Dhan & Charu, 2014). The garlic (*Allium sativum*) therapeutic potential could be attributed to its various organosulfur compounds include anti-obesity (Yang et al., 2018), antidiabetic (Hosseini & Hosseinzadeh, 2015), anticoagulant (Mikaili et al., 2013), antihypertensive (Reid, 2016) and lowering cardiovascular disease risks (Bradley et al., 2016). Historically, ancient civilizations used garlic as for preventing infection (Indians); as performance enhancer (Romans and Greeks army and athletes); fever, headache, cholera, and diarrhea (China) (Khorshed et al., 2016). Garlic is rich in polysulfide (i.e., diallyl disulfide and diallyl trisulfide), that are metabolized to hydrolypolysulfide (RSnH), during the endogenous synthesis of the physiologically active H₂S (Benavides et al., 2007; Rose et al., 2008; Qian & Lancaster, 2013). Indeed, the sulphur compounds (such as H₂S and polysulphides) have recently been discovered for their active involvement in controlling cellular systems, such as vasodilation and inflammation (Martelli et al., 2012; Zhang et al., 2017).

Due to garlic's beneficial effects to augment the production of these gasotransmitters, synthetic donors of these gaseous mediators have been developed. However, previous reports suggested that excessive generation of H₂S from synthetic sources (i.e., sodium sulphides, NaHS) can elevate the risk for ischemic stroke, Parkinson's and Alzheimer's diseases (Trevisani et al., 2005). Garlic administration is considered to be a safe dietary precursor for enhancing the generation of endogenous H₂S and NO through its potent polysulphide compounds (Benavides et al., 2007; Zhao et al., 2014). It is hypothesized that consuming garlic-derived organopolysulphides may have the potential to safely lower blood pressure (BP) (Benavides et al., 2007) and attenuate fatigue-related physiological responses during exercise (Veeranki & Tyagi, 2015), ultimately leading to improved exercise capacity. Interestingly, a new method for enriching the polysulphide content of garlic was discussed in previous study by Tocmo and colleagues in 2017. It appears that the boiling duration of 6 to 10 minutes can maximally enrich garlic's major

organopolysulphide compounds and is expected to augment its H₂S-generating capacity (Tocmo et al., 2017).

However, it is currently unknown whether polysulphide-enriched garlic (PEG), can improve H₂S production and elicit its therapeutic benefits in humans. Considering the potential of PEG to increase H₂S-releasing capacity and, in turn, positively influence a wide range of physiological processes (including vascular pressure and energy metabolism), investigations that examine the efficacy of PEG administration on BP and its physiological responses to exercise intervention are warranted. Therefore, the aim of this study was to investigate the acute effects of PEG supplementation on selected physiological responses and exercise tolerance in collegiate-level athletes.

METHODS

Participants

Twelve male collegiate-level athletes voluntarily participated in this study. Participants were recruited from among the university students and staff community of Universiti Teknologi MARA via email, instant messaging (i.e., WhatsApp) and printed poster advertisement. A set of exclusion were used to determine subject's eligibility which includes; 1) smokers; 2) consume dietary supplements (excluding macronutrients) or medicines within the past 4 weeks; 3) actively on suppositories – including over-the-counter pharmaceutical substances; 4) blood transfusion recipient within 1 month prior to screening; 5) any investigational research agent recipient prior to 30 days of experimental of experimental trial; 6) allergic or experienced to any garlic and/or H₂S related medication/supplement side effects; and 7) individual with the medical histories of drug dependence or alcoholism. The participants were required to record their dietary consumption and physical activity prior to the first experimental testing visit and were requested to execute the

same activities and meals intake in the subsequent visit. Participants were required to abstain from vigorous exercise a day prior to each visit to the testing centre. Additionally, participants were instructed to refrain from drinks containing caffeine and alcohol for 6-hour and 24-hour prior to each laboratory visit, respectively. Written informed consent were obtained from each participant after they were thoroughly briefed of the study protocol, including the possible risks and benefits of participation. Ethical approval was granted from the Research Ethical Committee of UiTM [REC/01/2020 (FB/2)] and this study was conducted in accordance with the Declaration of Helsinki.

Experimental Protocol

The participants reported to the testing centre on 2 different visits prior to supplementation testing. In the first visit, participants completed an incremental ramp test to assess participant's $\dot{V}O_{2max}$ and the gas exchange threshold (GET) for determination of exercise intensity ergometer's work-rate at 70% Δ (see measurements) (Lansley et al., 2011). In a second visit, pre-supplementation (PRE) resting BP and eH₂S was recorded, and a familiarization session of constant load exercise was performed by each participant. Subsequently, participants were asked to be present at the laboratory on a further two occasions to complete a high-intensity constant-load exercise following consumption of either 4 g of PEG or PLA (microcrystalline cellulose) for 5 days. The supplementation conditions were administered in a randomized, crossover, counter balanced-designed study with a 14 days of washout period between the experimental visits. On day 1 to 4 of each supplementation period, an equal dose of the supplement was given to the participants to be taken twice a day in the morning (2 g) and the evening (2 g). Participants then, were given entirely a single dose (4 g) of supplement following arrival to the laboratory on day 5 to be taken exactly 3-hour prior to the resting BP recording (Figure 1A). Resting eH₂S level were assessed immediately prior to the commencement of exercise trial. Participants' blood [lactate], and blood [glucose] were also assessed immediately before the start of the baseline cycling test (baseline), at

4 mins of the cycling exercise, at volitional fatigue and post-exercise (at 15-min) (Figure 1B) (Bailey et al., 2009).

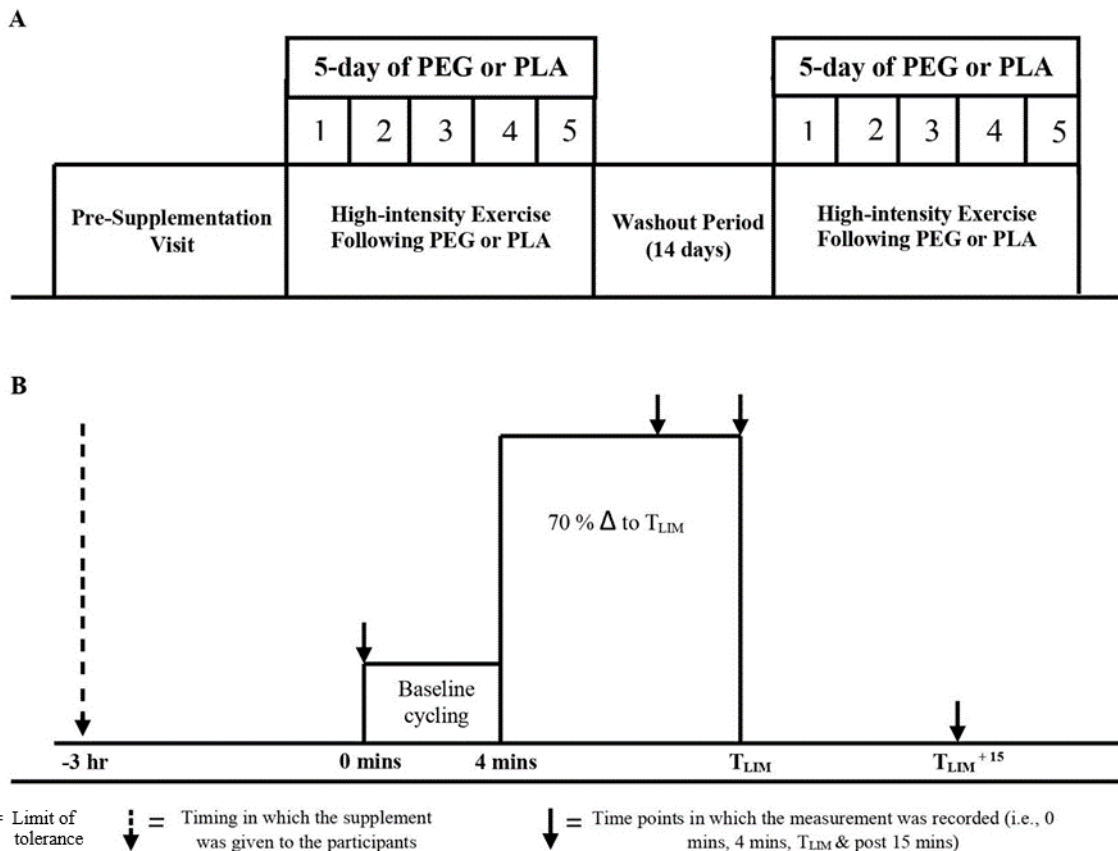


Figure 1: Illustration of the schematic crossover design of the study (A) and the exercise protocol utilized for the supplementation visits (B).

Supplementation Preparation

Raw garlic was acquired from local markets at Seremban, Negeri Sembilan. Voucher's specimens of the plant were identified (by trained botanists) and were deposited at Institutional Herbarium-Botanical Laboratory. The garlic bulb was weighted, crushed and boiled for a period of time to optimally enhance the polysulfide content as described in detail by Tocmo et al., (2017). The PEG

was then frozen at -80°C . The supplements were prepared in capsule form, with peppermint-scented oil apply to the outside of the capsules to make the supplementation conditions homogenous in both taste and smell (Bloch et al., 2013).

Measurements

Determination of Maximal Oxygen Uptake and Gas Exchange Threshold

The pre-supplementation exercise test started with baseline cycling of approximately 4 mins at 20 Watts, at which the rate of work intensity augmented at a rate of $30 \text{ Watts}\cdot\text{min}^{-1}$. During the test, participants were asked to cycle within 70–80 revolutions per minute (rpm) of pedal cadence to limit of tolerance (T_{LIM}). The test was stopped if the participant failing to sustain designated pedal cadence of $>70 \text{ rpm}$ for approximately 10 seconds. Each participant's saddle and handlebar configurations were replicated in each subsequent experimental trial. An electronically braked cycle ergometer was used to perform all the exercise testing (Lode Excalibur, Lode, The Netherlands). Pulmonary gas exchange was continuously measured and recorded on a breath-by-breath extraction method throughout the incremental tests. The highest 30 secs mean value recorded prior to the participant's volitional fatigue was taken as $\dot{V}O_{2\text{max}}$ and GET was determined by a combination of 3 different methods; 1) the modified V-slope; 2) the excess CO_2 ; and 3) the ventilatory equivalent (VE) (Bailey et al., 2009). The intensity for high-intensity constant-load exercise during experimental visits was calculated as the approximate work-rate at the GET and subsequent addition of 70% of the difference between the work-rates at the GET and $\dot{V}O_{2\text{max}}$ ($70\%\Delta$) (Lansley et al., 2011).

High-Intensity Constant Load Exercise

Participants performed baseline cycling for 4 mins at 20 Watts, with the intensity abruptly increased to individual work-rate of $70\%\Delta$. Participants were asked to cycle at a constant pedal of 70–80 rpm to the participant's limit of tolerance. The test was terminated up to the point in which

participant failing to sustain designated pedal cadence (>70 rpm) for more than 10 secs. The time-to-exhaustion was taken to represent T_{LIM} (Bailey et al., 2009).

Resting blood pressure

The BP was recorded via an automated sphygmomanometer (Evolv HEM-7600T, Omron Healthcare, Inc., Kyoto, Japan) in a seated position. Following arrival at the testing centre, participants were placed in a quiet room, with a thermoneutral temperature of $\sim 22^{\circ}\text{C}$ for 10 mins rest. There were 5 measurements recorded, and the mean of the final 4 recordings was used for the BP (systolic BP, diastolic BP and mean arterial pressure) analysis. The MAP was computed as $\frac{1}{3}$ x systolic pressure + $\frac{2}{3}$ x diastolic BP (Wylie, 2016).

Exhaled Hydrogen Sulfide (eH₂S)

For eH₂S level, participants carried out a slow maneuverer of vital capacity into 3L Tedlar reservoir bags (Dalian Haide Technology, Dalian, China) and, at the end of the maneuverer, the reservoir was sealed and analysed for eH₂S using the Interscan 4170-1999b (Interscan Corp, Simi Valley, California) (Toombs et al., 2010).

Blood Glucose and Blood Lactate

Blood samples were collected from the side of the fingertip with an auto-let lancet device Model No. OWAT0271 (Owen Mumford Ltd., Oxford, UK). Blood [glucose] and blood [lactate] was performed using a blood glucometer (One Touch [®] Ultra [®] 2, LifeScan Inc., CA, USA) and a blood lactate monitor (StatStrip, Model 1.0, Nova Biomedical, MA, USA). The validity and reliability of the blood glucometer and blood lactate monitor was verified before its use by comparing values from automated blood lactate and glucose analyser (YSI 2300 Stat Plus, Yellow Springs Instruments, OH, USA). For duplicate samples of blood lactate and glucose using above mentioned procedures, the CV were 1.5% and 1.8% respectively.

Macronutrient Analysis and Physical Activity Diary

Energy intake and physical activity routine (i.e., 24-hour) prior to the first experimental visit was assessed using a 24-hour self-recorded food and physical activity diary. Participants then, were asked to replicate such dietary habit and physical activity routine in all subsequent visits. Participants were thoroughly explained the appropriate method to record the diary prior to the start of the experiment and instructed to write down everything participants ate and drank (breakfast, lunch, dinner), consisting of details such as time of meals, food or drink consumed and portion or serving of the food or drink. The dietary macronutrient and overall energy intake were estimated (by accredited dietitians) Nutritics[®] diet analysis software version 4.2 (Nutritics Ltd., Co. Dublin, Ireland) with calorie guidelines reference from the Ministry of Health (Malaysia) was used to insert the food information which was missing from the software database (Ministry of Health Malaysia, 2016).

Statistical Analysis

A one-way ANOVA with repeated measures was used to compare the average macronutrient intake in each experimental condition. A repeated-measure ANOVA (one-way) was utilized to probe for differences between experimental condition (PRE, PLA and PEG) in resting BP and eH₂S data. Differences in T_{LIM} between the supplementation conditions (PEG and PLA) was analysed using a two-tailed, paired-samples *t*-test. Differences across the supplementation conditions and the appropriate time-points in blood [glucose] and blood [lactate] were assessed using two-way ANOVA with repeated measures. Further scrutiny of the differences was performed using post-hoc with the Bonferroni adjustment method. The data analysis was conducted using the GraphPad Prism software (version 8.1.2, GraphPad Software Inc., La Jolla, California, USA), with the statistical significance accepted at $p < 0.05$. Data presented as mean \pm SD.

RESULTS

The post survey indicated that each participant adhered to both supplementation course. No differences in total energy and macronutrient intake a day prior to the PRE, PEG and PLA condition (PEG vs. PLA: Energy (kcal) 2669.1 ± 572.3 vs. 2682.9 ± 589.0 ; Carbohydrate (g) 269.1 ± 57.04 vs. 264.0 ± 53.16 ; Protein (g) 177.8 ± 33.71 vs. 177.0 ± 29.80 ; Fat (g) 99.80 ± 32.44 vs. 103.8 ± 40.64). Further, no apparent variations were found in the duration spent on physical activities 24-hour prior to each trial experimental visit (PLA: 119 ± 6.2 hours, and PEG: 121 ± 7.3 hours).

Change in Blood Pressure Response

The systolic BP was significantly lower in the PEG group (120.5 ± 8.02 mmHg) compared to the PLA (124.3 ± 8.95 mmHg) and PRE (123.9 ± 8.59 mmHg) groups (both $p < 0.05$; Figure 2A). Similarly, mean arterial pressure was reduced in the PEG (88.7 ± 5.13 mmHg) compared to the PLA (90.6 ± 5.86 mmHg) and PRE (90.8 ± 5.55 mmHg) (both $p < 0.05$; Figure 2B), with no significant differences between diastolic BP in the PRE and PLA trials ($p > 0.05$).

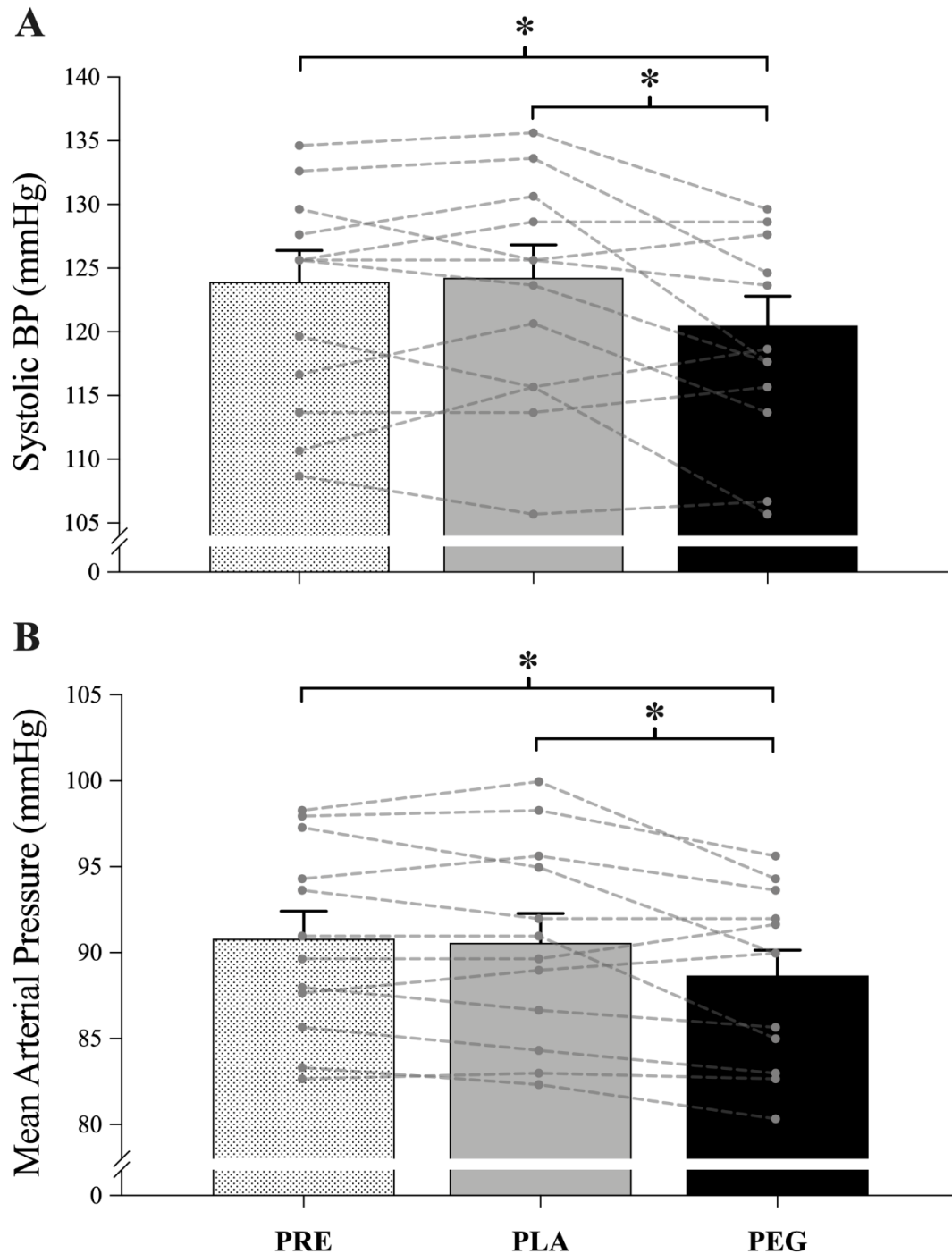


Figure 2: Mean \pm SEM of systolic BP (A) and mean arterial pressure (B) in the PRE, PLA and PEG condition. The dashed lines indicate individual responses. *Significantly different ($p < 0.05$).

Change in Exhaled Hydrogen Sulphide

The PEG significantly increased eH₂S level (4.6 ± 0.7 ppb) compared to the PRE (2.9 ± 1.0 ppb) and PLA (3.1 ± 1.0 ppb) conditions (both $p < 0.05$) (Figure 3).

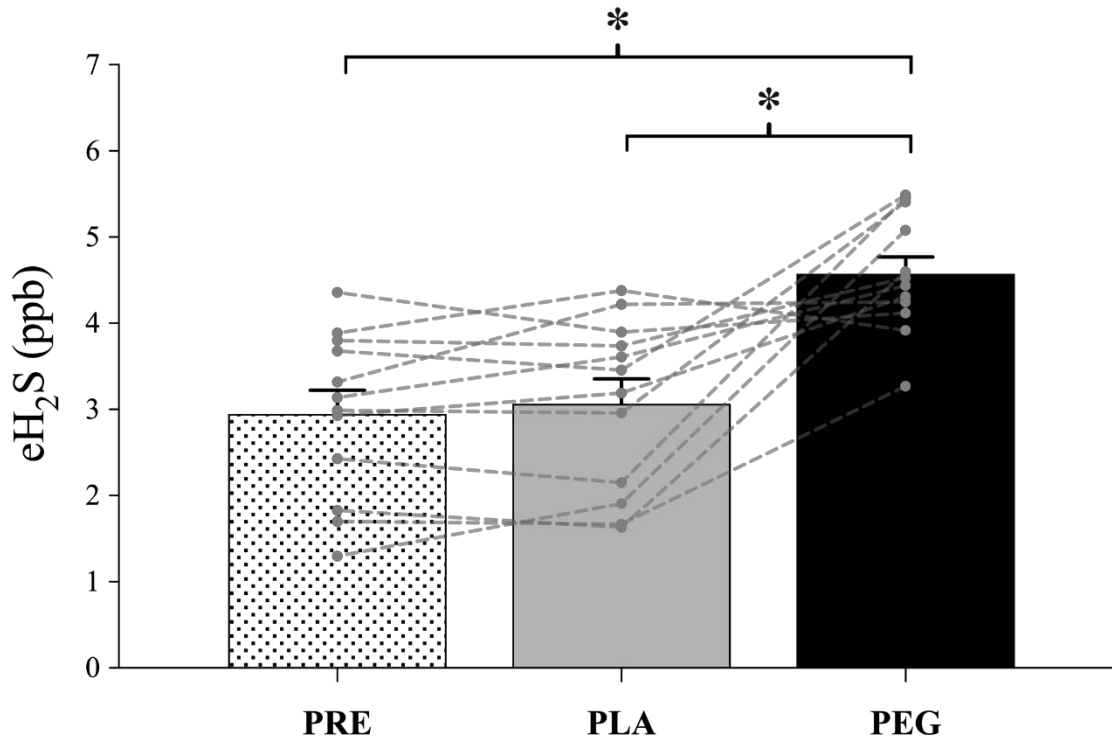


Figure 3: Mean \pm SEM of resting eH₂S in the PRE, PLA and PEG condition. The dashed lines indicate individual responses. *Significantly different ($p < 0.05$).

Change in Blood [glucose] and Blood [lactate]

During the exercise trial, PEG (4.08 ± 0.92 mmol/L) significantly lowered blood [glucose] compared to PLA (4.83 ± 0.79 mmol/L) ($p < 0.05$). However, no significant differences in blood [lactate] compared to PLA ($p > 0.05$) (Table 1).

Table 1: Mean \pm SD in blood [glucose] and blood [lactate] measured at different time points during high-intensity constant-load exercise in the PLA and PEG condition.

Variables	Time	PLA	PEG
Blood [glucose]	Rest	4.81 \pm 0.75	4.44 \pm 0.56
	4 mins	4.83 \pm 0.79	4.08 \pm 0.92*
	T _{LIM}	4.75 \pm 0.85	4.32 \pm 1.01
	Post 15 mins	5.08 \pm 0.84	4.59 \pm 1.24
Blood [lactate]	Rest	0.68 \pm 0.21	0.74 \pm 0.25
	4 mins	5.32 \pm 1.54	5.42 \pm 1.45
	T _{LIM}	9.08 \pm 1.88	9.86 \pm 2.50
	Post 15 mins	5.66 \pm 2.43	5.74 \pm 2.10

*Significantly different, $p < 0.05$.

Exercise Tolerance

T_{LIM} was not significantly affected by PEG compared to PLA conditions ($p > 0.05$). However, the trends observed ~6% improvement in time-to-exhaustion following PEG administration compared to the PLA. Further, a substantial improvement was apparent in majority of the participant, with 8 out of 12 participants had average T_{LIM} of +5.7% (Figure 4).

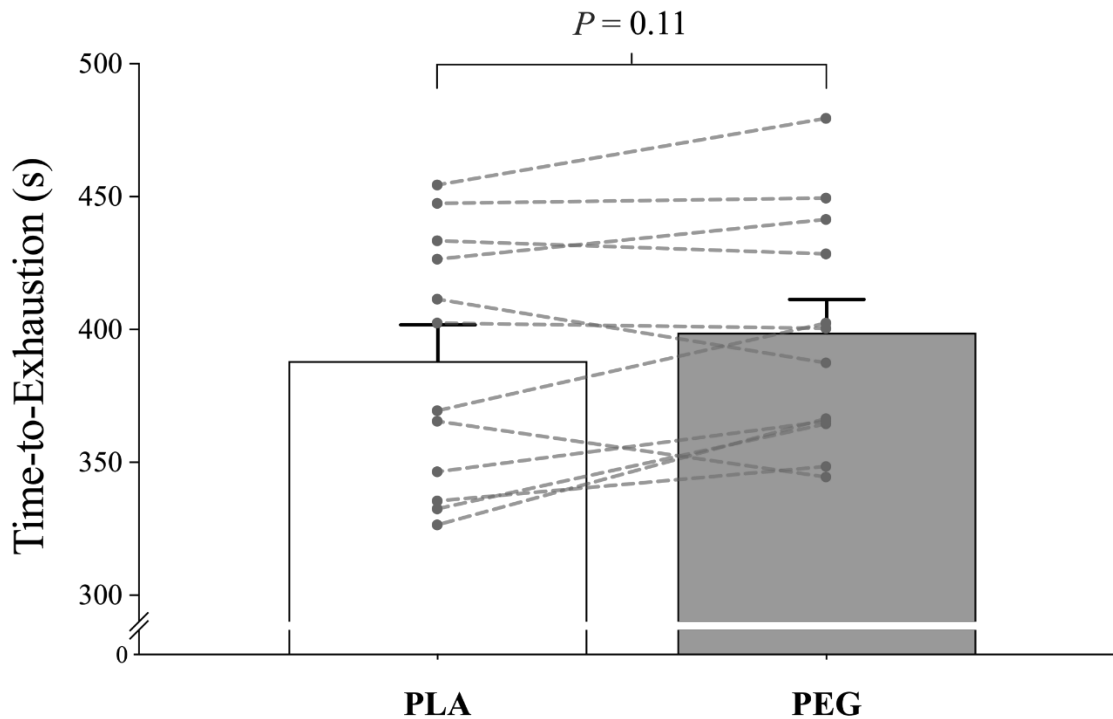


Figure 4: Mean \pm SEM of T_{LIM} in the PLA and PEG condition. The dashed lines indicate individual responses.

DISCUSSION

The purpose of current work is to investigate the effect of short-term (5-day) PEG supplementation on selected physiological responses to continuous, high-intensity exercise tolerance in collegiate-level athletes. Ingestion of PEG was well tolerated by all participants as evident by the lack of severe adverse events reported during the trial. The most frequent reactions to oral raw garlic consumption were odour ($n = 2$) with the PEG administered. This study observation noted that no moderate or severe adverse reactions to PEG were observed at 4 g, (the dose equivalent to an average-sized garlic clove), as reported by the subjects during final day of the supplementation period (day 5).

Polysulphide compounds such as diallyl disulfide (DADS) and diallyl trisulfide (DATS) isolated from fresh garlic are reported to be viable donor of H₂S in vitro (Benavides et al., 2007). Recent study have shown that moderate boiling of garlic homogenates enriches garlic's linear polysulphides production, including DADS and DATS which is expected to elevate garlic's H₂S - releasing capacity (Tocmo et al., 2017). The polysulfide content of garlic has long been speculated as the reason for the beneficial effect of garlic, likely via increasing bioavailability of H₂S levels. In current study, we report for the first time the improvement of H₂S level with short-term PEG supplementation in healthy human adult. The improvement of this physiologically relevant gaseous signalling molecule likely explained the BP-lowering effect reported in current work. This outcome is in line with elegant in vitro work by Benavides and colleagues which suggests that organopolysulphides compound, such as DATS and DADS, could be viable H₂S donors. The data from Benavides's study indicates that the garlic's polysulphides compounds (i.e., DATS and DADS) elevated H₂S production which then diffuses through plasma membranes causing vasorelaxation of intact aorta ring of an animal model (Benavides et al., 2007). Similarly, a previous experimental study reported that garlic intake resulted in a dose dependent decrease of BP in animal models (Nwokocha et al., 2011).

As for BP, MAP is a significant in the assessment of BP due to the facts that it measures the pressure required for adequate perfusion of the organs of the body. It is considered by many to be a better indication of perfusion than systolic BP. In current study, while PEG lowered systolic BP and mean arterial pressure after 5 days of supplementation, no significant changes on diastolic BP was observed in current study. The reason why remains unclear. However, some authors suggested that obvious diastolic BP reduction may be more pronounce in individual with higher baseline BP (Sjostrom et al., 2015). This may be related to the fact that the population has a lower level of H₂S bioavailability compared to their healthy counterparts (Al-Magableh et al., 2015). Since current study recruited normotensive, highly active adult, this may affect the magnitude of the reduction in diastolic BP. Speculatively, the length of the duration of supplementation by which garlic supplementation could induce its hypotensive effect on diastolic BP should be considered. Several previous studies have shown the potential of garlic supplementation in reducing diastolic

BP when supplementation lasted longer than 2 weeks (Ried & Fakler, 2014; Ried et al., 2008). Therefore, it is possible that significant reduction could be seen in diastolic BP when the duration of PEG supplementation is longer.

Short-term PEG supplementation in current dosage does not significantly improve T_{LIM} compared to PLA. This is contrary to the previous studies which observed potential improvement in exercise tolerance (Morihara et al., 2006; Veeranki & Tyagi, 2015). Notably, however, there is an upward trend of T_{LIM} following PEG administration. Specifically, 8 out of the 12 participants in the present study demonstrated substantially improved T_{LIM} following PEG (improvement of ~6%), with the other four having a slight decrement in exercise capacity (~3%). We speculated that substantial inter-individual differences in the responses to PEG may result from individual's baseline H_2S level and polysulphide metabolism.

Garlic has been reported to alter the substrate metabolism such as reduction in blood [glucose] (Jelodar et al., 2005) and blood [lactate] (Hwang et al., 2019). Current study observed that short-term supplementation of 4 g of PEG significantly lowers blood [glucose] during T_{LIM} (at 4 mins iso-time). It is possible that the improvement in some participants during high-intensity constant load exercise could be attributed to the finding of attenuated blood [glucose] in PEG group. This phenomenon may indicate enhance sparing of muscle glycogen utilisation during exercise with PEG. While the current study used a simple enzyme-based electrochemical finger-prick glucose meter, future research could assess muscle glycogen content via biopsy of skeletal muscle tissue to ascertain the impact of PEG supplementation to alter muscle glucose response during exercise. In contrast, blood [lactate] was not significantly altered by orally administered PEG. Such disparity may be a result from different lactate metabolism in humans, compared to animal model (Connor et al., 1982).

Given the physiological benefits afforded by H_2S , and the concern for the safety of synthetic/inorganic H_2S donors like sodium hydrosulphide (a fast donor of H_2S , which tend to produce excessive H_2S levels), there is a demand for a safe natural source of bioavailable H_2S for

therapeutics purposes. Current work shows, for the first time, that the PEG supplementation may be a viable natural alternative to enhance H₂S bioavailability *in vivo*.

CONCLUSION

The short-term (5-day) supplementation of PEG lower resting BP in this investigation, likely in response to improvement in H₂S bioavailability as observed in significant elevation of eH₂S level. This study reported, for the first time, that enrichment of garlic polysulphides via moderate boiling could augment the H₂S level in human. This milestone is significant considering synthetic/inorganic H₂S donors is associated with toxicity risk leading to serious health concerns. PEG supplementation, in current study, did not measurably impact on high-intensity constant load exercise. However, the effects were highly variable between subjects, which requires further study to elucidate the factors that influence PEG ergogenic potential.

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