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POLYSULFIDE-ENRICHED GARLIC SUPPLEMENTATION IMPROVES COGNITIVE FUNCTION AND REDUCES HEART RATE DURING HIGH INTENSITY INTERMITTENT EXERCISE

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ABSTRACT

This study aim is to determine the effect of short-term (5-day) polysulphide-enriched garlic (PEG) administration on cognitive function and physiological responses to high-intensity intermittent exercise performance. Fifteen recreational team-sport players 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 in day-5 prior to the completion of a Yo–Yo intermittent recovery level 1 test (YYIRTL1). Resting exhaled hydrogen sulphide was 42% greater, while systolic BP and mean arterial pressure was lower by 3% in PEG compared to PLA ($p < 0.05$). Performance in the cognitive function test was 4% and 3% faster at rest and during YYIRTL1, respectively, with performance in YYIRTL1 only tended to increase in PEG compared to PLA ($p = 0.08$). Mean heart rate was lower during YYIRTL1 ($p < 0.05$). These results indicate that PEG supplementation, with dose equivalent to average sized garlic clove, lower both resting BP and heart rate during exercise. Short term PEG supplementation may also enhance cognitive function during high-intensity intermittent performance.

Keywords: *Garlic, Supplementation, Cognitive, Exercise Performance, Ergogenic*

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INTRODUCTION

Garlic (*Allium sativum L.*) is one of the most popular condiments and seasonings worldwide. For thousands of years, the benefits of garlic have been recognized, especially for its purported therapeutic benefits. Garlic's biological effects are attributed to its distinctive organosulphur compounds. Emerging data indicate that some plant, especially from the onion genus family, has the potential to be a donor for important signaling molecule hydrogen sulfide (H_2S) synthesis. H_2S is regulates several physiological processes that are important to exercise performance, including vasodilation, mitochondrial respiration and skeletal muscle contractility (Bonaventura et al., 2010; Bradley et al., 2016; Kabil et al., 2014). However, previous reports suggest that excessive generation of H_2S from synthetic sources (e.g., sodium sulphide salt) may increase the risk of severe hypotension, cerebral stroke, haemorrhagic shock, inflammatory diseases and mental retardation (Zhang et al., 2017). Since the physiological advantages offered by H_2S are numerous, the scientific community has shown significant interest in the search for a safe dietary donor of H_2S. Previous reports noted that garlic and garlic-derived organopolysulphides can be alternative agents for safely augmenting endogenous H_2S levels (Benavides et al., 2007; Li & Lancaster, 2013; Rose et al., 2018).

It is acknowledge that different garlic preparation methods may result in varying polysulfide profile, and thus H_2S releasing capacity (Lawson & Hughes, 1992; Tocmo et al., 2017). There is some evidence to indicate that garlic and garlic-derived polysulfides to be effective agent to lowers BP (Bradley et al., 2016), reduce cardiac workload during exercise (Verma et al., 2005), and improve cognitive function in complex activity tests (Kimura, 2015). Furthermore, several reports have also documented supplementation with garlic can positively impact both direct (e.g., time to fatigue in exercise test) and indirect (e.g., blood markers of muscle fatigue) markers of performance during intense exercise (Morihara et al., 2007).

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Interestingly, the process of boiling garlic for a short, predetermined time can enrich the concentration of polysulphides, thus increasing the H_2S-releasing activity of garlic (Tocmo et al., 2016). It has been observed that administration of garlic polysulphides improved H_2S-releasing activity likely impacted the vasodilatory activity as evident by BP reduction *in vitro* (Benavides et al., 2007; Kanagy et al., 2017). Accordingly, an increase in vasorelaxation during exercise may lead to sustained adenosine triphosphate availability through the enhancement of hydrogen ion outflow (Joyner & Casey, 2015) and this has been hypothesised to possibly impacted exercise capacity (Veeranki & Tyagi, 2015). Lower heart rate which indicates the vasodilation effect, were believed to affect the cognitive function via improvement in brain haemodynamic. However, the influence of polysulphide-enriched garlic (PEG) consumption on physiological responses in humans has yet to be examined. The purpose of this study was to assess the physiological and performance effects of short-term (5-day) PEG supplementation on intense intermittent exercise. We hypothesized that PEG supplementation would lower the heart rate (HR) and improve cognitive function during exercise, and increase the distance covered in the YYIRTL1.

METHODS

Participants

Fifteen recreational male team-sport players were recruited to complete the experimental study. The participants were required to record their dietary consumption and physical activity in the first experimental trial and were requested to execute the same activities and meals intake in the subsequent experimental trial. Participants were asked to refrain from any caffeine and alcohol intake 6- and 24-hour prior to each experimental 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. All

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procedures in this study were approved by Research Ethical Committee of UiTM [REC/01/2020 (FB/1)] and in accordance with the Helsinki Declaration of 2008.

Experimental Protocol

The participants were asked to be present at the testing centre (at 9:00 – 10:00 73 a.m.) on 4 different visits within eight weeks. On two different days (at least 72 hours apart), participants were familiarized and subsequently tested for a standard YYIRTL1 until volitional exhaustion (Krustrup et al. 2003). Specifically, during the first visit, participants were required to perform the 5 minutes of YYIRTL1 test to familiarize with the experimental procedures (cognitive task) to be employed during the main test. On the second visit, after 72-hour rest period, the standard YYIRTL1 test is completed until volitional exhaustion. The total distance covered in the YYIRTL1 test in visit two was recorded to calculate participant's 70%$_{max}$ (70% of the distance relative to participant volitional exhaustion), which served as timepoint during YYIRTL1 for reaction time test (as a measure for cognitive function test) assessment in the experimental visits. This modified protocol of YYIRTL1 has been adopted from (Thompson et al., 2016).

Following the pre-experimental visit, participants were subsequently assigned via randomized, crossover, balanced study design to receive the PEG (4 g) and PLA (microcrystalline cellulose) for five days, with a 14-day washout period. On day one to four of each supplementation period, participants went to the laboratory and were given an equal dose of the supplement to be taken in the morning (2 g) and the evening (2 g). Following participants' arrival to the laboratory on day-5, participants were given the supplement to be taken entirely in a single dose (4 g), exactly 3-hour prior to the BP recording (first experimental assessment) (Figure 1A). Resting eH_2S level was assessed immediately before the start of YYIRTL1 (resting baseline data). Participants' cognitive performance was evaluated immediately before (resting baseline), and during the YYIRTL1 (at 70% max and volitional exhaustion). Additionally, heart rate was recorded continuously throughout the entire YYIRTL1 (Figure 1B).

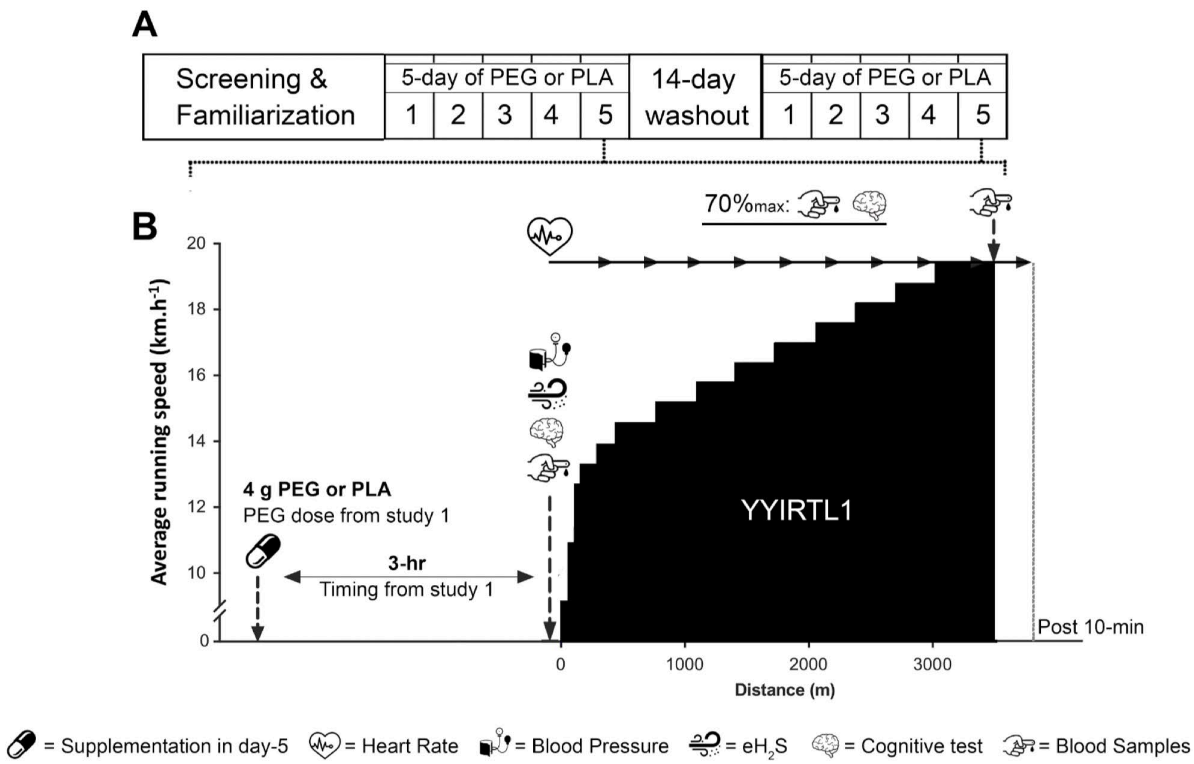

Figure 1: Schematic representation of the crossover design (a), and the assessment performed following 5-day of PEG and PLA supplementation (B).

Supplementation Preparation

Raw garlic was obtained in Seremban, Negeri Sembilan, from local markets. The voucher specimens of the plant were identified and deposited in the Herbarium-Botanical Laboratory. As reported in detail by Tocmo et al., (2017) the garlic bulb was weighed, crushed, and boiled for period of time to best enhance the polysulfide concentration. The PEG was then frozen at a temperature of -20°C. The supplements were produced in capsule form, with peppermint-scented oil applied to the capsules' exteriors to ensure that the supplementing conditions were consistent in terms of taste and odor (Bloch et al., 2013).

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Measurements

High Intensity Intermittent Exercise Test

The YYIRTL1 test requires all participants to perform 40 m (2 × 20 m) shuttle runs at speeds signalled by audio beeps, followed by short 10 seconds active recovery at 5 m designated area behind the starting line. During the test, participants were requested to briefly stop at $70\%_{max}$ (70% of maximum total distance of YYIRTL1 during pre-experimental visit) for participant's reaction time test (see justification for $70\%_{max}$ in Pre-experimental Visit) (Thompson et al., 2016). Following the $70\%_{max}$, participants continue the YYIRTL1 test until volitional exhaustion, or when participants are incapable of maintaining the designated running speed. Participants were withdrawn from the test after failing to reach the finishing line twice, with cumulative distance recorded. The enumerator recorded the final distance completed by the participant and estimates participants maximal aerobic capacity using the mathematical equation by Bangsbo et al. (2008).

Resting Blood Pressure

BP was recorded via an automated sphygmomanometer (Evolv HEM-7600T, Omron Healthcare, Inc., Kyoto, Japan) a sitting position. Following arrival at the testing centre, participants were placed in a quiet room, with a thermoneutral temperature of ~22°C for 10 minutes rest. There were five measurements, and the mean of the final four recordings was used for the analysis. The MAP was computed as ⅓ x systolic pressure + ⅔ x diastolic BP (John Wylie, 2016).

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Exhaled Hydrogen Sulphides (eH_2S)

Participants carried out a slow maneuverer of vital capacity into 3L Tedlar reservoir bags (Dalian Haide Technology, Dalian, China) without holding their breath. Using an Interscan 4170-1999b portable H_2S gas detector (Interscan Corp, Simi Valley, California), at the end of the maneuverer and then analysed for eH_2S. Exhaled air will then be channeled to the sampling ports of the detectors. The sample pump flow is regulated by an adjustable voltage regulator and was set to 1 $L \cdot min^{-1}$ (Toombs et al., 2010).

Cognitive Function Test

Participants were required to complete a Stroop test for assessment of cognitive performance. The Stroop test was conducted using Inquisit® (version 4.0, Millisecond Software, WA, USA) as described in (Thompson et al., 2016). Briefly, participants were instructed to react to a sequence of text strings using a custom keyboard as rapidly and precisely as possible (Haskell et al., 2012). The accuracy response and reaction time was recorded with duration of 90 seconds on each Stroop test.

Heart Rate Response

Heart rate (HR) was measured continuously starting from 5-min prior to exercise intervention until 10 mins following the termination of YYIRTL1 using short-range heart rate telemetry and Bluetooth 4.0 receiver (HRM Dual, Garmin International Inc., Garmin Ltd.). The HR response to exercise was modelled to measure indirect cardiac output dynamics over time. Heart rate data was time-aligned, ensemble-averaged for each time point and converted to percentage relative to age-predicted maximum heart rate (%HR_{max}).

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Statistical Analysis

A one-way repeated measures ANOVA was used to determine the difference in average macronutrient intake, a day prior to each experimental condition. A one-way repeated measures ANOVA was also used to probe for differences in resting eH_2S, and BP indices. Differences in YYIRTL1 test performance between the supplementation conditions (4 g PEG and PLA) was analysed using a two-tailed, paired-samples t-test. Differences across the supplementation conditions (PEG, PLA and PRE) and across the appropriate time-points in reaction time (as a measure of cognitive function) and heart rate response was analysed using two-way repeated measures ANOVA. Analysis of data was conducted using the GraphPad Prism software (version 8.3.0, GraphPad Software Inc., San Diego, California, USA), with statistical significance was accepted at $p < 0.05$. Data were reported as mean and standard deviation (mean ± SD) except for figures (mean and standard error mean; mean ± SEM).

RESULTS

The post survey indicated that each participant adhered to both supplementation course. No differences in energy macronutrient intake a day prior to the first PEG and PLA trials (PEG vs. PLA: energy (Kcal) 2533 ± 690.1 vs. 2506 ± 660.9; carbohydrate (g) 267.3 ± 67.09 vs. 263.61 ± 56.82; protein (g) 174.9 ± 40.75 vs. 170.0 ± 29.68; fat (g) 87.70 ± 31.95 vs. 88.42 ± 37.77). Similarly, there are no apparent variations in duration spent on physical activities 24-hour prior to each trial experimental visit (PLA: 119 ± 6.2 hours, and PEG 4 g: 121 ± 7.3 hours). No significant side effect from both supplementations (PLA and PEG) was reported.

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Changes in Exhaled Hydrogen Sulfide (eH_2S)

The PEG significantly elevates eH_2S by PEG (4.1 ± 1.6 ppb) compared to the PLA (2.9 ± 1.2 ppb) and PRE (3.0 ± 1.2 ppb) conditions (both $p < 0.05$) (Figure 2).

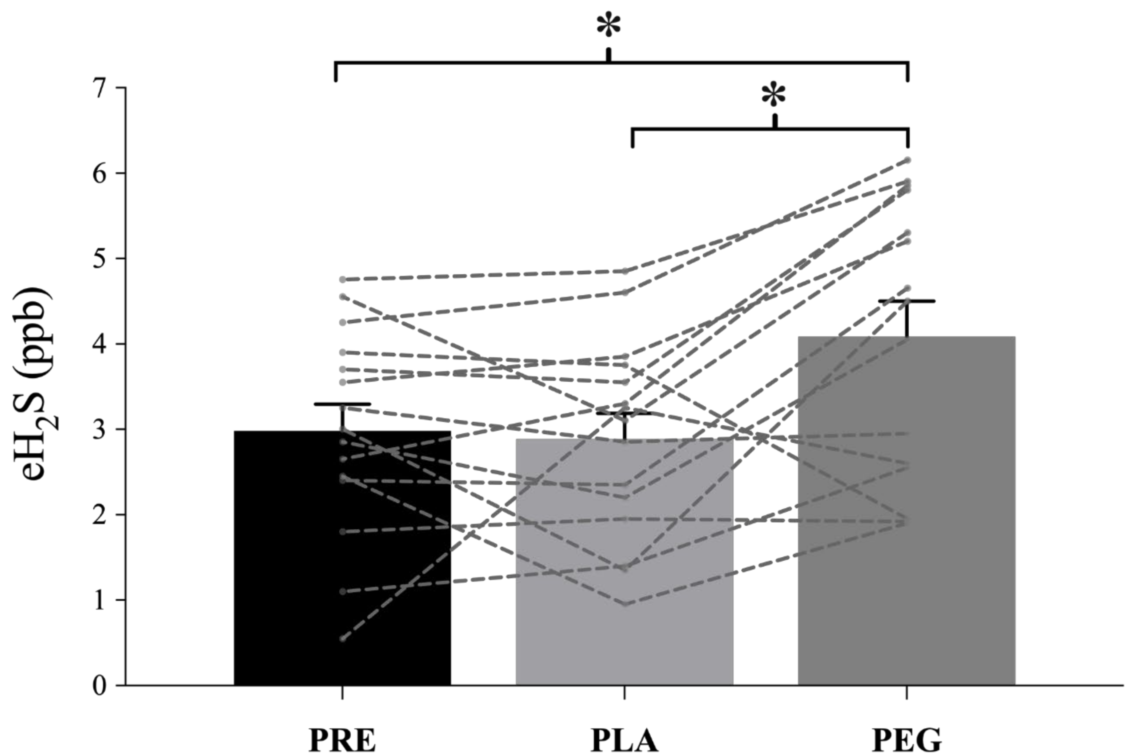

*Figure 2: Resting exhaled hydrogen sulphide in the PRE, PLA and PEG. the dashed lines indicate individual responses. *Significantly different (p < 0.05)*

Changes in Blood Pressure Indices

The Systolic BP was significantly lower in the PEG group (122 ± 6 mmHg) compared to the PLA (125 ± 7 mmHg) and PRE (124 ± 6 mmHg) groups ($p < 0.05$; Fig. 2). Mean arterial pressure (MAP) was reduced in the PEG group (89 ± 4 mmHg) compared to the PLA (91 ± 5 mmHg) and PRE (90 ± 5 mmHg) groups ($p < 0.05$), with no significant differences between diastolic BP in the placebo and control trials ($p > 0.05$) (Figure 3).

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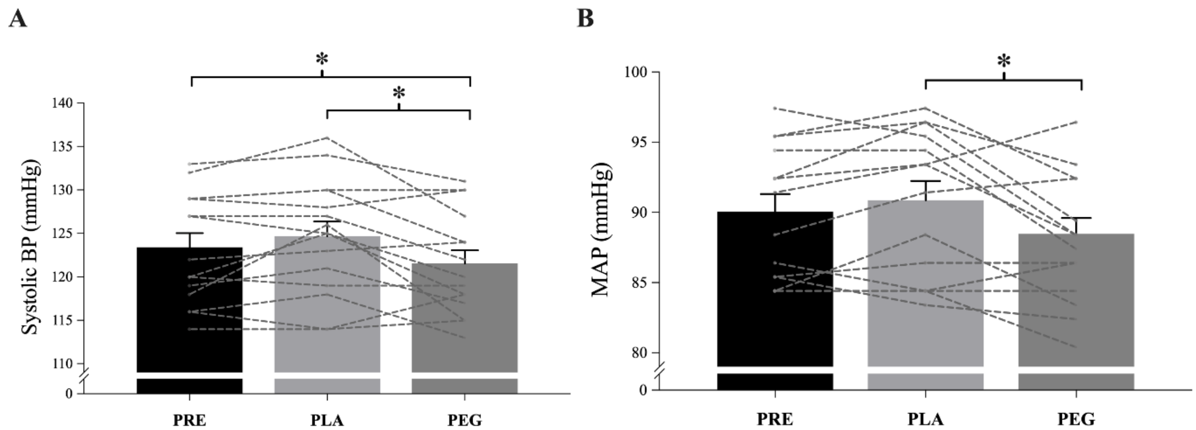

Figure 3: Systolic BP (A) and mean arterial pressure (B) in the PRE, PLA and PEG condition. The dashed *lines indicate individual responses. *Significantly different (p < 0.05).*

Changes in Cognitive Function

PEG supplementation significantly improves cognitive function at rest in PEG (699± 75 s) compared to PLA (727 ± 85 s) and at 70%$_{max}$, PEG (693 ± 73 s) compared to PLA (712 ± 82 s) (p < 0.05; Fig. 5). Despite no significant differences in reaction time performance at exhaustion during PEG compared to PLA, there is a notable trend toward significance difference between the supplementation conditions (p = 0.092) (Figure 4).

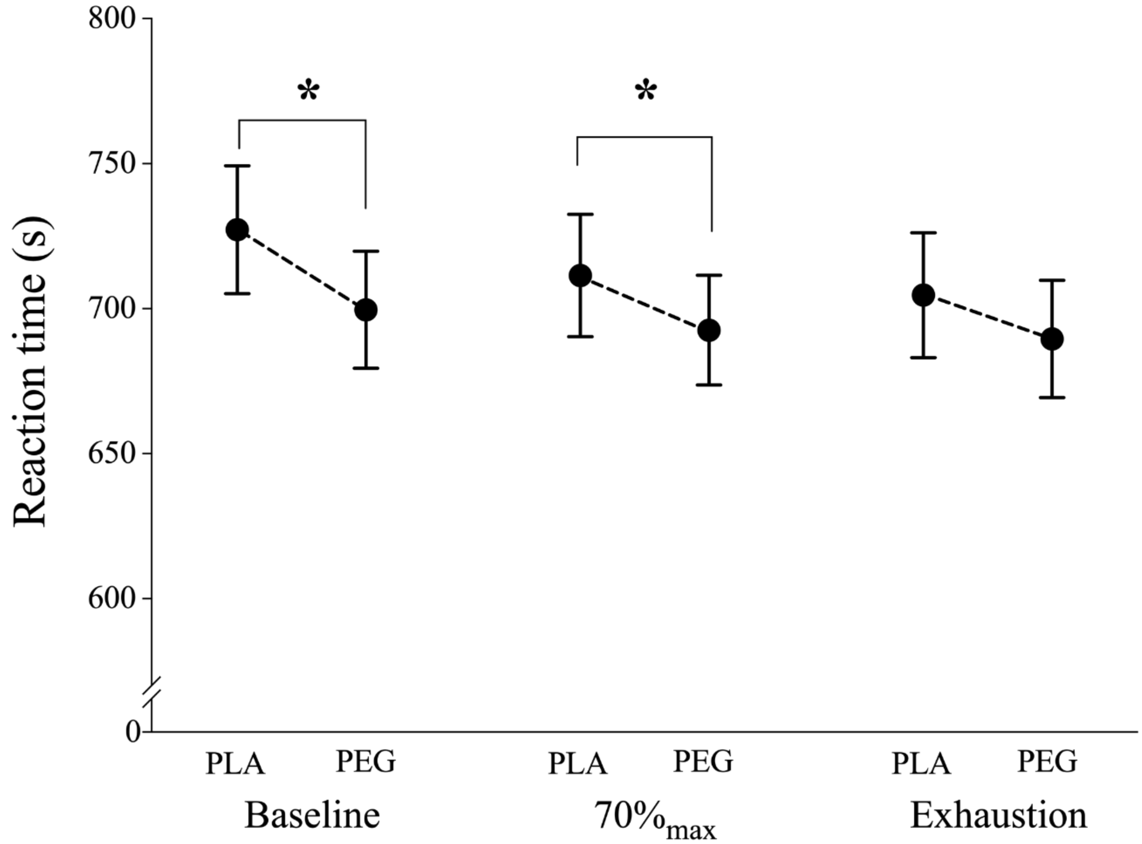

Figure 4: Change in cognitive performance measured as reaction time during Stroop test at PRE and following PLA and PEG supplementation. *Significantly different ($p < 0.05$).

Heart Rate Response

During exercise trial, PEG significantly lowered HR at 6 mins (90.7 ± 3.3 $\%HR_{max}$), 9 mins (93.4 ± 3.0 $\%HR_{max}$), 10 mins (94.1 ± 3.9 $\%HR_{max}$) and at $70\%_{max}$ (89.87 ± 2.70 $\%HR_{max}$) compared to PLA (Figure 5). No significant difference in $\%HR_{max}$ during rest, at exhaustion and 10 min after termination of YYIRTL1 between PEG and PLA condition ($p > 0.05$).

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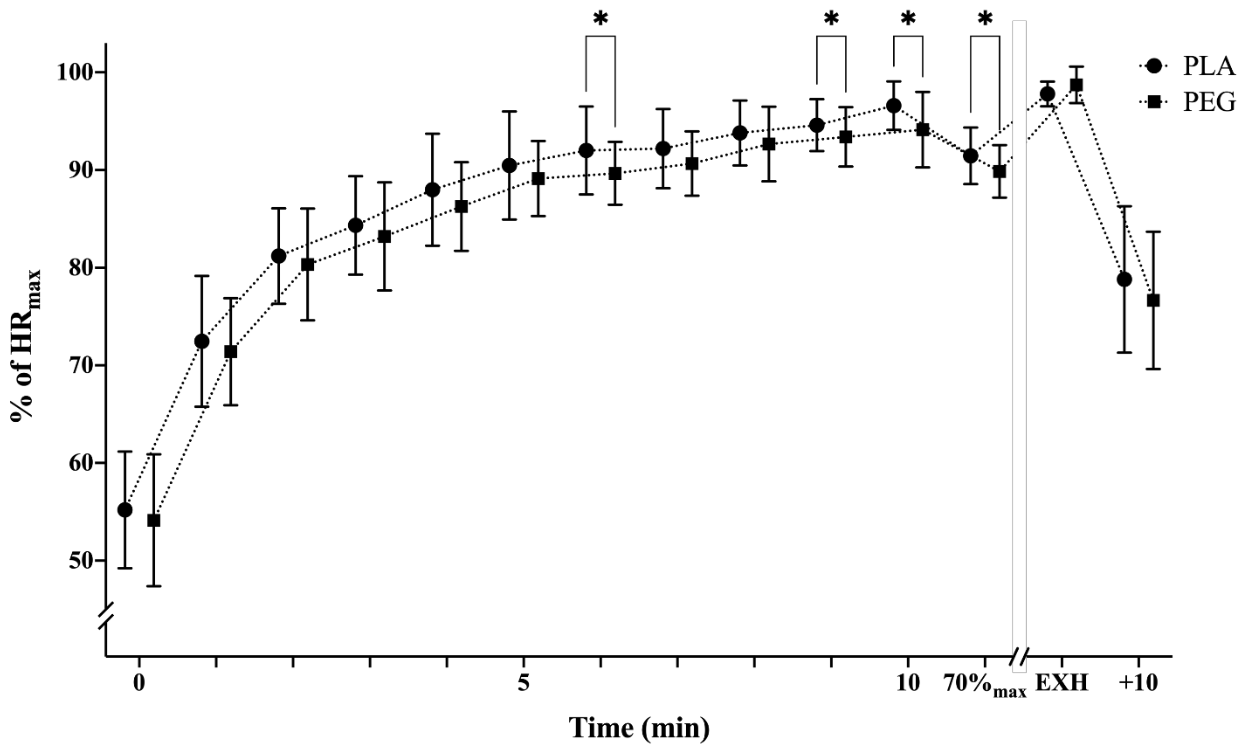

Figure 5: Percentage of heart rate maximum at rest (%HR_{max}), during and at 10 min in YYIRTL1. *Significantly different ($p < 0.05$). Note: pairwise data is arranged in interleaved fashion for each time-point.

Changes in Exercise Performance

Although no different in distance covered was observed in PEG (1517 ± 314.8 m) compared to PLA (1477 ± 373.2 m), a trend toward significant level was observed between condition (p = 0.08) (Figure 6).

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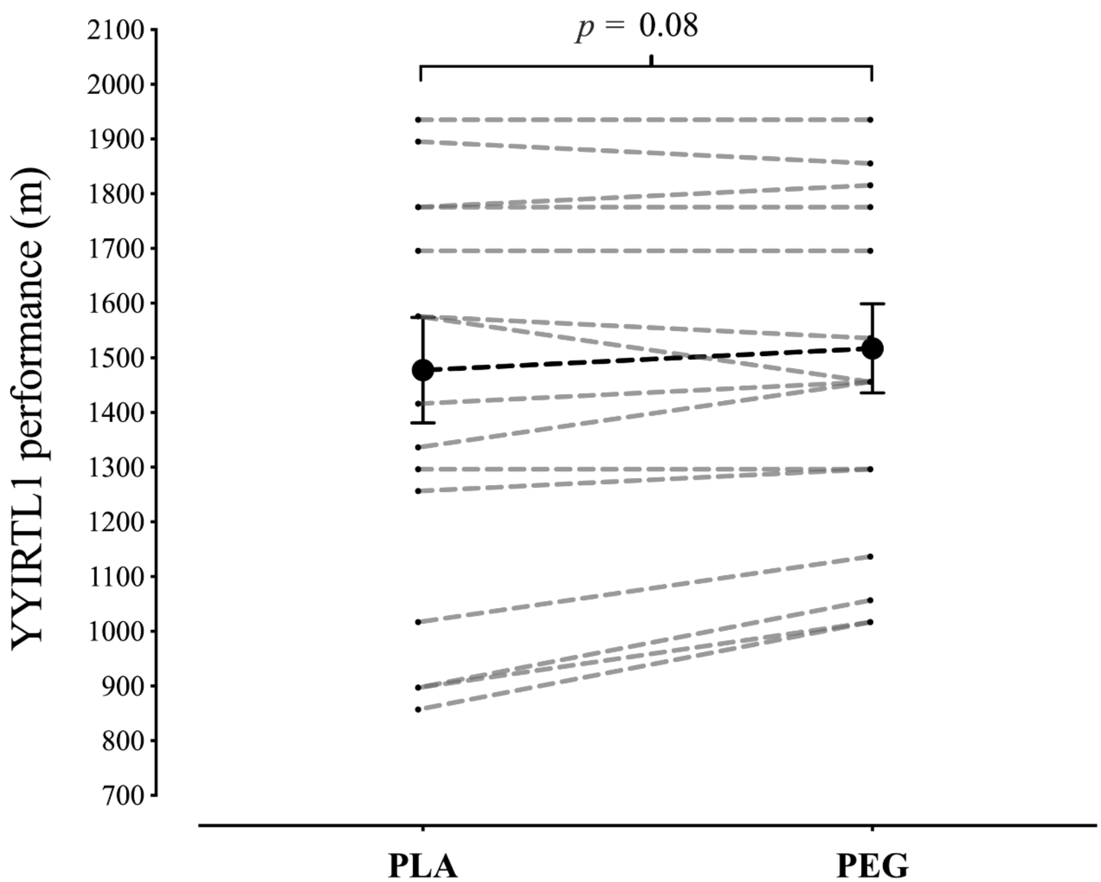

Figure 6: Change in cognitive performance measured as reaction time during Stroop test at PRE and following PLA and PEG supplementation.

DISCUSSION

This is the first investigation to assess the physiological and performance effects of short-term (5-day) PEG supplementation on intense intermittent exercise. Both supplementations were deemed well-tolerated as no major adverse effects reported throughout the trial. The principal original findings were that short term PEG supplementation lower both resting BP and enhanced cognitive function and lowers the cardiac workload (heart rate) during high-intensity intermittent performance. Notably, while the overall 3% improvement in total distance in

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YYIRTL1 following PEG supplementation was not statistically significant, appreciable trend towards improvement was evident in current study.

It was suggested earlier that the allyl polysulphides from garlic (i.e., DADS and DATS) exhibited H_2S-releasing capacity in both in vitro and animal-based studies (Benavides et al., 2007; Predmore et al., 2012). Principally, crushed or ruptured cell of garlic layers allows the conversion of allin to allicin, which ultimately converted into allyl polysulphides. In support of garlic capability to enhance bioavailability of H_2S, Benavides and colleagues in 2007 has shown that garlic derived polysulphide DATS leads to significant increases in persulphide, which then reacts further with thiols to produce H2S. The researchers show that an individual erythrocyte instantaneously induce garlic-derived organopolysulphides into hydrogen sulphide H_2S, which then relaxes intact aorta rings. Provided that these findings are applicable to humans, the enhanced eH_2S might be reflective of endogenous production of H_2S in cellular level that lowers BP and may improve exercise performance. The level of allyl polysulphides, however, is rather insignificant in regular size garlic cloves and may not be enough to bring about the desired therapeutics changes (Jepson et al., 2013). However, study by Tocmo et al. (2017) brings about the possibility of enriching the polysulphides compounds with a rather simple procedure moderate boiling process. Enriching garlic polysulphides in this Tocmo' s study have shown to be viable H_2S donors that could increase H_2S-releasing capacity (Benavides et al., 2007; Predmore et al., 2012). Further, the evidence presented in DeLeon's study also supports the idea that garlic to be a viable donor of H_2S. The author shows that garlic oil, which is rich in DATS, could improve the bioavailability of the H_2S and result in enhanced myocardial contractile function in animal models (Deleon et al., 2016).

Current study found that systolic BP significantly reduced by ~2.6% with no changes in diastolic BP following 4 g PEG compared to PLA. The reason why a reduction was only seen in systolic BP, but not diastolic BP remains unclear. However, it was suggested that reduction in diastolic BP may only be elicited in the clinical population with apparent vascular issues. This may be related to the fact that the population has lower levels of H_2S compared to their healthy counterparts. Several previous studies had shown garlic's potential in reducing diastolic BP after a chronic supplementation longer than 2 weeks (Bradley et al., 2016; Schwingshackl et al., 2016). Further study looking at inter-subject response to PEG is needed to further elucidate the factor involved through which polysulphide-rich supplementation may exert its antihypertensive potential.

The findings from current study indicate that PEG supplementation improves the cognitive function in reaction time test at rest and during exercise, but not at exhaustion point during the high-intensity intermittent exercise. This observation was consistent with Behera et al. (2019) who reported improved cognitive function (memory) in older mice receiving the garlic compound (allyl sulfide) garlic supplementation. We speculated that, the efficacy of PEG may have similar (or greater) effect for its greater H_2S-releasing capacity. H_2S is a gaseous signaling molecule which physiological role involved neuromodulation (Rose et al., 2018) and therefore, the change of cerebral blood flow with respect to cognitive task could be speculated. The improved cerebral haemodynamic response may draw a distinction in reaction time between PEG and PLA in the present study.

The heart rate responses were found to be significantly lower during the test with PEG compared to PLA. In accordance with the present, study by Verma and colleagues demonstrated garlic oil administration, which is expected to be rich in polysulphides content, improved cardiac performance and exercise tolerance in the clinical population (Verma et al., 2005). In detail, Verma and colleagues reported garlic significantly reduced heart rate at peak exercise ($p < 0.01$) and the research team proposed that low heart rate may impose lower workload on the heart, resulting in better exercise tolerance. Therefore, administration of a type of garlic with high level of polysulphides, such as garlic oil and PEG, appears to be a practical ergogenic strategy, partly through improving heart workload (as evident in lowering of heart rate) during exercise.

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Despite the physiological advantages of H_2S, there is concern for the safety of synthetic/inorganic H_2S donors, such as sodium hydrosulphide (a fast donor of H_2S which tends to produce excessive H_2S levels), therefore, it is essential to seek a safe and natural source of bioavailable H_2S for health. In comparison, unlike the long-term administration of inorganic sulphide, organopolysulphide supplementations (at least in the current acute study) do not seem to accelerate any development of serious adverse events such as acute hypertension, septic shock or stroke (Santhosha et al., 2013). The hypotensive effects of PEG in the current study are comparable to those administered with angiotensin converting enzyme inhibitors (ACEI), beta-blockers and angiotensin II type 1 receptor antagonists (Ried et al., 2008). PEG is rich in polysulphide contents that can potentially function as safe and slow releasing H_2S donors, eliciting the therapeutic advantages associated with H_2S.

CONCLUSION

The short-term (5-day) supplementation of PEG lower resting BP, and enhanced cognitive function and lowers the cardiac workload (heart rate) during high-intensity intermittent performance. This study reported, for the first time, that enrichment of garlic polysulphides via moderate boiling could augment the H_2S level in human, and likely the physiological improvement observed in current work. This milestone is significant considering synthetic H_2S donors is associated with toxicity risk leading to serious health concerns such as severe hypotension, cerebral stroke, haemorrhagic shock, and mental retardation. While PEG supplementation, did not significantly impact the high-intensity intermittent exercise in current study a strong trend towards improvement in distance covered in YYIRTL1. Further study is requires to elucidate the factors that influence PEG ergogenic potential.

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