

# EFFECT OF A NITRIC OXIDE PRE-WORKOUT SUPPLEMENT ON MUSCULAR ENDURANCE

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## ABSTRACT

*Introduction:* Multi-ingredient dietary supplements have been developed to increase nitric oxide (NO) production, with the expectation of improving resistance training performance. Many of these supplements contain ingredients and/or ingredient amounts that have yet to be studied for their synergy or efficacy in increasing NO production and thus, resistance training performance.

*Purpose of the study:* A randomized crossover design was used to investigate the effect of a citrulline malate (CM) based non-stimulant nitric oxide pre-workout supplement (NOPWS) blend or placebo on Young Men's Christian Association (YMCA) bench press performance.

*Applied Methodology:* Thirty-minutes were provided between NOPWS or placebo ingestion and YMCA bench press assessment. Pre/post heart rate was taken following each condition. Two- and one-way repeated measures ANOVAs ( $\alpha = 0.05$ ) were run to determine the effect of each condition on heart rate (HR) and the number of repetitions performed (respectively) during the bench press test.

*Achieved major results:* There was a significant two-way interaction between the treatment and time for HR,  $F(1, 20) = 6.82, p = .017$ . Resting HR was significantly higher during the supplement session ( $M = 74.67, SE = 2.54$  bpm) than during the placebo session ( $M = 69.14, SE = 2.31$  bpm),  $F(1, 20) = 8.19, p = .010, \eta_p^2 = .290$ . No significant difference was found between conditions for number of repetitions performed.

*Leads:* A specific CM-containing NOPWS blend had no significant effect on a standardized assessment of upper body muscular endurance.

*Practical implications:* These findings highlight the need for consideration of the minimum effective dosage and assimilation timing of each respective ingredient when developing or researching pre-workout supplement blends.

*Originality/Value:* This is the first known research to study the effectiveness of this specific blend of ingredients on resistance training performance. Thus, this study provides necessary foundational knowledge for future research in this area.

**Keywords:** Nitric oxide, YMCA bench press, supplement, citrulline malate, pre-workout

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## INTRODUCTION

Nitric oxide (NO) is a lipid-soluble gas that is produced in multiple locations within the body including the endothelial cells of blood vessels (Schwedhelm et al., 2007). The vasoactive nature of NO has the potential to cause vasodilation of arterial and venous blood vessels, thereby promoting an augmentation

of blood flow to skeletal muscle tissues. An increase in blood flow provides an opportunity for enhanced oxygenation of muscular tissues and clearance of exercise metabolism byproducts. Increases in NO availability have also been shown to influence skeletal muscle contractility, glucose uptake, and mitochondrial activity (Campos et al., 2018; Gonzalez &

Trexler, 2020; Stamler & Meissner, 2001), all of which are important components of performance during exercise.

Research has suggested that NO synthesis can be enhanced through exogenous means, via food sources (e.g., dark green leafy vegetables; beet root juice) and supplementation (Jones et al., 2020; Gough et al., 2021). Nitric oxide production from exogenous sources occurs in the endothelial cells via the L-arginine-NO pathway, where L-arginine is converted to NO and L-citrulline. (Gonzalez & Trexler, 2020; Sunderland et al., 2011). Supplemental L-citrulline can bypass many metabolic processes and be directly converted to L-arginine (Gonzalez & Trexler, 2020; Van de Poll et al., 2007; Windmueller & Spaeth, 1981; Wu & Morris, 1998) which is a precursor to NO synthesis. Therein, supplementation with L-citrulline is a common method utilized to enhance NO synthesis. L-citrulline may also suppress arginase activity, thereby assisting in the bioavailability of L-arginine for NO synthesis (Bailey et al., 2015; El-Bassossy et al., 2012; Gonzalez & Trexler, 2020; Morita et al., 2014). Thus, L-citrulline supplementation can potentially enhance NO production through multiple mechanisms.

Supplementary L-citrulline is often combined with malate, which is a tricarboxylic acid intermediate. Malate has been purported to increase the rate of ATP production (Bendahan et al., 2002; Bescos et al., 2012; Gonzalez & Trexler, 2020) and mitigate lactic acid production (Gonzalez & Trexler, 2020; Wax et al., 2016). Malate supplementation may also increase the effectiveness of the malate aspartate shuttle in exercising musculature, thereby leading to increased efficiency in mitochondrial respiration and, potentially, energy utilization (Agudelo et al. 2019; Gough et al., 2021; Wu et al., 2007). Although the exact mechanisms have yet to be scientifically defined, it

is believed that these two ingredients work synergistically by improving skeletal muscle perfusion dynamics, which can then improve the ATP production and utilization cycle (Gonzalez & Trexler, 2020).

The use of citrulline malate (CM) as an exercise supplement is well studied, but the findings are highly inconsistent. Several authors have found CM improve resistance training performance. Perez-Guisado et al. (2010) found an acute dose of 8-grams of CM improved the number of repetitions performed by 52.92% in a pectoral training session (including bench press) of 41 men. Similarly, Wax et al. (2016) found a significant increase in the number of repetitions performed in the number of chin-ups, reverse chin-ups, and push-up repetitions performed (respectively) after an acute dose of 8-grams of CM.

Several other authors have failed to find a significant change in resistance training performance after use of CM. For instance, Gonzalez et al. (2018) investigated the effect of an acute dose of 8-grams of CM on multi-set bench press repetition performance. The authors did not find any consistent evidence CM was effective at improving bench press performance. Though an isokinetic exercise regimen was utilized, Chappell et al. (2018) also failed to find a significant difference in the number of repetitions performed in the knee extension exercise using an acute dose of 8-grams of CM in 15 men and women.

Similar to the use of L-citrulline and malate together, combining ingredients is a common practice in the supplement industry. Therein, many pre-workout supplements (PWS) are blends of many ingredients are designed to work independently and/or synergistically to amplify the efficacy of the blend. Though CM is commonly used in supplementation and research, CM-based PWS blends have been sparsely studied and yielded unclear findings.

For instance, Bergstrom et al., 2018, utilized a PWS containing 18-primary ingredients, including 6-grams of CM and 350 mg of caffeine. The authors found a significant increase in the volume of exercise performed by the lower body. However, the authors were unable to distinguish if the increases were due to the high amount of caffeine in the PWS or a combination of the other active ingredients (including CM). The PWS utilized in Bergstrom et al. (2018) highlighted a common problem in researching many caffeine-containing supplement blends, Specifically, it is difficult to identify if performance improvements were due to the stimulatory nature of such compounds or the synergistic (or singular) effects of the other ingredients.

However, there are supplement blends that have been created to modulate NO activity with the expectation of improving performance. For instance, a current commercially available, non-stimulant NO-supporting pre-workout supplement (NOPWS; Hype Reloaded®) has an active ingredient list consisting of CM, glycerol monostearate, alpha-GPC, icariin, potassium, and L-norvaline. Glycerol has the potential to improve aerobic and anaerobic performance (Patlar et al., 2012) and potentially increase hydration levels when combined with hyperhydration protocols (Lyons & Riedesel, 1993; Magal et al., 2003; Wingo et al., 2004). However, glycerol monostearate is the glycerol ester of stearic acid and utilized in the current study as an emulsifier to maintain the freshness and consistency of the NOPWS.

Another active ingredient in the NOPWS is Alpha-GPC, which can serve as a source of choline for acetylcholine production (Kawamura et al., 2012; Gage et al., 2021). Repeated muscular contractions, performed at a high rate, can reduce the amount of acetylcholine available to the neuromuscular junc-

tion, thereby – potentially – reducing exercise duration and/or performance. However, the effectiveness of alpha-GPC in improving exercise performance parameters is mixed. For instance, Ziegenfuss et al. (2008) found an increase in peak bench press force (compared to placebo) after supplementation of 600-mg of alpha-GPC, 90-minutes before assessment. However, Parker et al. (2015) tested the effects of 400-mg of Alpha -GPC, 30-minutes before assessment of mood, reaction time, hand-eye coordination, power, speed, and agility, in a group of young ( $22 \pm 3.4$  years) males and females. No significant difference was found between the Alpha-GPC and placebo conditions on any other physiological measure. Thus, alpha-GPC may be useful for improving certain parameters of resistance training performance, but more research is necessary.

Icariin is a flavonoid of *Herba epimedii*, that has been traditionally studied for use in treating erectile dysfunction (Chen et al., 2014; Low & Tan, 2007; He et al., 2021), but has yet to be studied for its usefulness in improving resistance training assessment or performance. Still yet, in research involving in-vitro human endothelial cells, icariin was found to significantly enhance bioactive NO (Xu et al., 2007). These findings indicate icariin may have an indirect avenue for increasing NO production, but the research is too meager for any definitive suggestions made for the purposes of supplementation. L-norvaline is a non-proteinogenic unbranched-chain amino acid and potent arginase inhibitor (Gilinsky et al., 2020; Rognstad, 1977). Arginase is an enzyme that converts L-arginine to L-ornithine and urea (Gilinsky et al., 2020). Increased arginase activity can inhibit NO production by regulating L-arginine availability (Durante et al., 2007; Gilinsky et al., 2020). Several authors (Abhijit De et al., 2016; El-Bassossy et al., 2013; Gilinsky et al., 2020; Romero et al., 2008) have

found L-norvaline supplementation to be beneficial for indirectly increasing NO bioavailability through a reduction in arginase activity. Reduced arginase activity through L-norvaline supplementation has also been shown to improve acetylcholine-stimulated NO generation (El-Bassossy et al., 2013; Romero et al., 2008), which could provide an avenue for the justification of a potential synergistic effect with alpha-GPC. However, each of these studies were performed on rats and no muscular performance-based research is known to be available in human studies.

On their own respective merit, the additional active ingredients found in the Hype Reloaded® NOPWS (glycerol monostearate, alpha-GPC, icariin, potassium, and L-norvaline) have been either sparsely studied in their relationship to resistance training or, to these author's awareness, not at all in humans. Therein, simply studying the effects of an ingredient such as icariin on resistance training performance would make a significant contribution to the scientific body of literature. Furthermore, with exception of CM, the synergistic expectations from combining these specific ingredients (in similarly specific amounts and ratios) have not been studied. Finally, the acute use (rather than a loading or daily regimen) of these ingredients on standardized tests of muscular endurance performance remains unknown. In addition to these unknowns, it is important for supplementation development to determine which ingredients work best together and if the dosage of a single ingredient should be altered based on expected synergistic outcomes. Therein, the primary purpose of this study was to determine the effects of a specific NOPWS blend on YMCA bench press performance in college-aged men and women. Secondarily, resting and post-exercise heart rate (HR) were measured to monitor potential HR changes associated with use of the supplement. We hypothesize the use of this

supplement will increase the number of repetitions performed over that of the placebo, in a group of participants who have experience with resistance training.

## METHODOLOGY

### *Participants*

This project and the procedures within were approved by the Tennessee State University institutional review board (HS-2020-4426). Participants ( $N = 21$ ) were recruited via flyer placed in the student wellness center of a university in the southeastern United States. Participants were also recruited by verbal announcements made in university health and kinesiology-based courses.

Participants consisted of healthy and injury-free males ( $N = 14$ ) and females ( $N = 7$ ) between the ages of 18 and 25. Each participant's ability to participate in exercise activity was verified by completion of the physical activity readiness questionnaire (PAR-Q). A reviewed and signed informed consent document was required for participation in the experiment. All participants were recreationally trained, which was operationally defined as participation in an average of 2 to 3 resistance training sessions per week in the last calendar year. To be included in the study, participants were asked to acknowledge their recreationally trained status via signing an informed consent document, while also verbally verifying this status with the primary investigator. Additionally, only participants who were able to verbally verify their ability to bench press at least 80 lbs (males) or 35 lbs (female) for at least 5-repetitions, were included in the experiment. As it were, no participant who began and/or completed the study (male or female) performed less than 16-reps during either testing session. Participant characteristics are listed in Table 1.

**Table 1.** *Participant descriptive characteristics*

<b>Characteristic</b>	<b>Mean</b>	<b>SD</b>
Age (years)	23.5	2.6
Height (cm)	174.8	8.2
Body mass (kg)	87.5	23.3

**General procedures**

An experimental, 2 x 2, randomized, double-blind, placebo-controlled cross-over design was utilized. The double-blind procedure was implemented with concealment of the supplement or placebo performed by an independent researcher and blinding revealed upon completion of the trial. Participants were assigned to either the treatment or placebo condition using a random number randomizer. Each participant reported to the laboratory on 2 separate occasions, at the same time of day, 5 days apart. Fatigue-inducing activity was prohibited for 48 hours and food consumption a minimum of 2 hours before each testing session. Caffeinated beverages and other forms of stimulants were prohibited for at least 4 hours prior to testing. A study-controlled diet or strategically specific diet was not required of participation, but participants were informed of a requirement to maintain their usual dietary and hydration habits through completion of their second and final testing session. Participants were also required to refrain from any vasodilating supplements such as beetroot juice and L-arginine, and/or supplements with ingredients used in the study's NOPWS (see Table 2), for at least 7 days prior to the first testing session.

During the first visit, each participant was asked to sit quietly while filling out the required paperwork (PAR-Q, informed consent). Anthropometric measures were then taken, and

the participant outfitted with a heart rate (HR) monitor strap. The participant was then asked to sit quietly for 1 minute to allow HR to move closer to a resting state, so a baseline measure could be taken. After 1 minute, the HR was recorded, and the participant was given either the placebo or supplement. The participant was then required to sit quietly for 15 minutes. After 15 minutes had passed, the participant began a standardized warm-up protocol, lasting approximately 10 minutes. Upon completion of the warm-up, a 5 minute preparation period was allotted for the participant to move to the testing bench and be instructed on the testing protocol. The metronome was started at 4:30 of this preparation period, whereby the participant had 30 seconds to begin the assessment. Per the manufacturer's recommendations and previous research (Jagim et al., 2016) indicating an improvement in upper body muscular endurance through use of multi-ingredient pre-workout blend, 30 minutes was provided for pre-assessment assimilation time. Heart rate was taken immediately after completion of the assessment and participants were prescribed a cool-down period of 5 minutes of walking and deep-breathing before being allowed to leave the testing facility. The same procedure was utilized for each participant's second visit, except anthropometric measures were omitted and the remaining condition tested.

**Table 2.** *Supplement facts*

<b>Ingredient</b>	<b>Amount per serving</b>
Citrulline malate (2:1)	6 g
Glycerol monostearate	2 g
Alpha GPC	300 mg
Icariin Horny Goat Weed (leaf) extract (std. to 20% Icariin)	150 mg
Potassium (as Potassium Chloride)	100 mg
L-Norvaline	100 mg

## SPECIFIC PROCEDURES

### *Anthropometric measures*

*Measurement of height:* Height was measured to the nearest tenth of a centimeter (cm) on the first day of testing, in bare feet, with a stadiometer (Health-O-Meter, Perform Better, Cranston, RI).

*Measurement of body mass:* Body mass was measured to the nearest tenth of a kilogram (kg) on the first day of testing, in bare feet, with a standard Physician's scale (Health-O-Meter, Perform Better, Cranston, RI).

### *Physiological measures*

*Measurement of HR:* Resting HR was measured with the Polar (Polar Electro, Kempele, Finland) H10 HR monitor chest strap, linked via Bluetooth to the investigator's Polar Flow application.

### *Bench press Assessment*

The YMCA bench press protocol was utilized for assessment of muscular endurance. The YMCA bench press test requires male and female participants to lift an 80- and 35-lb. barbell as many times as possible while maintaining a repetition pace set to a metronome cadence of 60 beats per minute. Male participants utilized a standard 45 lb. Olympic barbell and weight plates (up to 80 lbs.), while the female participants utilized a 35 lb. Olympic barbell (16). The YMCA bench press test was utilized due to its use a standardized assessment of upper body muscular endurance (need source). This assessment has been found to not only assess muscular endurance but has also been shown to be highly correlated to max bench press strength performance (Invergo et al., 1991; Kim et al., 2002) and was found to account for 86% of the variance in predicting bench press strength (Invergo et al., 1991).

*Bench press procedure:* The participant positioned themselves in a supine position on the bench. The spotter assisted the participant with lifting the bar off the rack and into a starting

position (arms fully extended above the chest). A research technician would start the metronome and within three beats the participant lowered the bar to their chest. Once the bar touched the participant's chest, the weight was required to come to a momentary complete stop (i.e., bouncing the bar off the chest was counted as a 'non-rep'), then the arms fully extended again on the ensuing metronome beat. This process was repeated until the participant was no longer able to complete a full repetition without assistance from the spotter or the participant was no longer able to keep pace with the required metronome cadence.

### *Supplement Specifics*

The NOPWS utilized (*Hype Reloaded*, Blackstone Labs™) contains a unique blend of 6 active ingredients (see Table 2). The supplement can be purchased in nutrition/supplement stores or ordered online (Blackstone labs.com). The supplement/placebo was mixed into an opaque cup with 8-oz. of water, per instructions from the supplement manufacturer. Fruit punch flavor was chosen for the supplement. While the placebo was a fruit-punch flavored non-nutritive, non-carbonated soft drink mix with a similar taste and consistency profile. To match the taste and consistency profile of each condition, 3 g of drink mix (standard serving size) was mixed with 8 oz. of water and the suggested supplement serving size, while 4.5 grams of drink mix and 8 oz. of water was used for the non-supplement beverage condition.

## STATISTICAL ANALYSIS

Data analysis was performed with SPSS (version 25). Descriptive statistics for participant information are expressed as mean  $\pm$  standard deviation. A two-way repeated measures ANOVA was run to determine the effect of NOPWS supplementation overtime on HR during the YMCA bench press test compared

to placebo. Significant interactions were followed by analysis of simple main effects.

A one-way repeated measures ANOVA was run to determine the effect of NOPWS on the number of reps performed on the YMCA.

**RESULTS**

Participant repetition and heart rate data can be found in table 3. A two-way repeated measures ANOVA was run to determine the effect of an NOPWS on HR compared to placebo. There were no outliers, as assessed by examination of studentized residuals for values greater than  $\pm 3$ . Pre/post HR data were normally distributed, as assessed by Shapiro-Wilk’s test of normality on the studentized residuals ( $p > .05$ ). There was a statistically significant two-way interaction between the treatment and time for HR,  $F(1, 20) = 6.82, p = .017$ .

Resting HR was significantly higher during the supplement session ( $M = 74.67, SE = 2.54$  bpm) than during the placebo session ( $M = 69.14, SE = 2.31$  bpm),  $F(1, 20) = 8.19, p = .010, \eta_p^2 = .290$ , a mean difference of 5.524 (95% CI, 1.497 to 9.551) bpm. Posttest HR was not significantly different between treat-

ment conditions. As would be expected there was a significant difference between resting HR ( $M = 69.14, SE = 2.31$ ) and posttest HR ( $M = 105.05, SE = 3.29$ ),  $F(1, 20) = 180.98, p < .001, \eta_p^2 = .900$  in the placebo condition as well as the supplement condition, resting HR ( $M = 74.667, SE = 2.539$ ) and posttest HR ( $M = 102.76, SE = 2.98$ ),  $F(1, 20) = 86.84, p < .001, \eta_p^2 = .813$ .

A one-way repeated measures ANOVA was run to determine the effect of a NOPWS on YMCA bench press performance. There were two outliers, but the repetition ranges for each were acceptable and thus included in the analysis. The data were normally distributed, as assessed by Shapiro-Wilk’s test of normality on the studentized residuals ( $p > .05$ ). The NOPWS did not elicit significant changes in the number of YMCA bench press repetitions performed when compared to the placebo condition,  $F(1, 20) = .84, p = .371, \eta_p^2 = .040$ .

ment conditions. As would be expected there was a significant difference between resting HR ( $M = 69.14, SE = 2.31$ ) and posttest HR ( $M = 105.05, SE = 3.29$ ),  $F(1, 20) = 180.98, p < .001, \eta_p^2 = .900$  in the placebo condition as well as the supplement condition, resting HR ( $M = 74.667, SE = 2.539$ ) and posttest HR ( $M = 102.76, SE = 2.98$ ),  $F(1, 20) = 86.84, p < .001, \eta_p^2 = .813$ .

**Table 3.** YMCA Bench press test repetition and HR results

	Placebo		Supplement	
	Mean	SD	Mean	SD
Pre HR	69.14	10.57	74.67*	11.63
Post HR	105.05**	15.08	102.76**	13.66
Reps	40.76	15.65	39.33	13.73

\*Significantly different from placebo. \*\*Significantly different from pre-test HR

**DISCUSSION**

This was the first known study to assess the usefulness of a NOPWS for improving single-set muscular endurance exercise (via the YMCA bench press test). The specific mix of ingredients utilized in this supplement are not known to have been scientifically studied. It was hypothesized that the supplement would

increase the number of repetitions performed over that of a placebo condition. However, the supplement failed to improve YMCA bench press performance when compared to the placebo condition.

The primary active ingredient in this NOPWS was CM. Previous research utilizing CM and/or a supplement containing CM, has shown

an improvement in upper body resistance training performance. For example, Glenn et al. (2017) found a significant increase in bench press performance (overall # of repetitions performed) following 6-sets to failure at 80% of 1-rep max. Similarly, Perez-Guisado & Jakeman, (2010) found a significant increase in the number of bench press repetitions performed by the CM condition, over 4 sets at 80% of 1 rep max following a pectoral training session. However, the current study only utilized one set to failure to test the effectiveness of the NOPWS. Therein, in support of Perez-Guisado & Jakeman, (2010) and Glenn et al. (2017), if only the first set (which was also performed to failure) was utilized for comparison, no significant difference was found between the CM and placebo conditions (i.e., all significant differences were found due to the change in the total multi-set training volume, not a single set comparison). These findings fall in line with those of Gonzalez et al. (2018) who did not find a significant difference between placebo and CM supplement conditions after completion of 1 or 5 sets of up to 15 repetitions or to failure at 75% of bench press 1 rep max. Similarly, though L-citrulline was used (rather than CM), Cutrufello et al. (2015), found no significant difference between conditions during 1 or 4 sets of chest press at 80% of 1 rep max. It should also be noted that each of the previously mentioned studies utilized a testing protocol with a resistance of anywhere from 75% to 80% of bench press 1 rep max or bodyweight (e.g., chin-ups). Comparatively, the current study utilized the much lower standardized resistance of the YMCA protocol. Thus, the current findings reflect and confirm that CM-based supplements do not increase the number of reps performed during a single set of upper body endurance exercise from body weight through 80% of 1 rep max.

Though a multi-set protocol may be

required to appropriately utilize CM or an NOPWS for resistance training performance enhancement, a look at the dosages of each ingredient may provide further explanation for the findings of the current study. The NOPWS used for this investigation contained 6-grams of CM. An acute dose of 6 gr of CM has been shown to increase plasma citrulline and arginine levels, and NO production (da Silva et al., 2017; Sureda et al., 2009; Sureda et al., 2010), but has yet to prove effective for improving any performance or recovery parameter studied (da Silva et al, 2017). Previous research (Perez-Guisado & Jakeman, 2010; Wax et al., 2016) has shown an improvement in resistance training performance with an acute dose of as little as 8-grams of CM, but these findings occurred via the result of multi-set assessment regimen, rather than the 1-set assessment utilized in the current study. Therefore, although, as little as 5.6 gr taken daily for a week has been shown to positively impact blood flow dynamics in otherwise healthy middle-aged men (Ochiai et al., 2012) it could be that a minimum of 8 g is required for single dose-related changes and/or to improve exercise performance over the course of the workout.

The supplement used for this study was a blend of many ingredients expected to work synergistically to improve performance. However much like the CM dosage, any expected singular or synergistic effect from the combined supplement ingredients may have been hampered by the supplied, per-serving, dosage of each ingredient.

For instance, in the limited research related to physical (i.e., non-cognitive) performance parameters, 600 mg of alpha-GPC was effective at improving peak bench press force by 14% compared to placebo (Zeigenfuss et al., 2008). However, the Hype Reloaded supplement only supplies 300 mg per serving. Icariin (horny goat weed) has been shown to



increase testosterone in rats at a human dosage equivalent of 200 mg/kg of bodyweight or roughly 900 mg of Icariin for a 150 lb. person. Yet, the studied supplement blend contains only supplies 150 mg per serving. Finally, in rats, at a dose of 50 mg per kg (roughly a 579 mg dose for an 80 kg, 182 cm male), L-Norvaline, has been shown to inhibit Arginase activity, which can help improve NO production and assist with acetylcholine response (El-Bassossy et al., 2013). However, the studied NOPWS contains only 100 mg of L-Norvaline per serving. Considered collectively, these findings cannot discount the synergistic capability of the blend of ingredients used in this study's NOPWS – when used at the appropriate dosages. However, it appears many of the supplied ingredients in this NOPWS are under-dosed compared to the effective dosages utilized in other research.

In respect to potential dosage complications with this NOPWS, an additional consideration should be given to the manufacturer's recommended ingestion timing. The current study provided 30 minutes between beverage consumption and the beginning of the YMCA assessment. The 30 minute time frame was chosen because this was the manufacturers recommendation, as listed on the supplement packaging. Yet, despite these manufacturer's recommendations, previous research utilizing similar ingredients (e.g., CM, Alpha-GPC) have allowed 60 to 90 minutes for supplement assimilation time (Perez-Guisado & Jakeman, 2010; Glenn 2017; Zeigenfuss, 2008). In contrast, research conducted by (Gonzalez, 2018) found no change (compared to placebo) in 5 sets of 75% 1 rep max bench press performance, from ingesting 8 grams of CM 40 minutes prior to testing. Consequently, though the current study strictly followed the manufacturers recommendations, future research utilizing similar ingredients should heavily

consider waiting a minimum of 60-minutes to allow for complete assimilation, regardless of the manufacturer's recommendations.

### ***Practical Applications***

Though great care was taken to ensure a high degree of internal validity, maintenance of a degree of practicality introduced certain limitations to the current study. First, no measures of supplement bioavailability were taken to support the mechanistic pathway thought to be responsible for vasodilation (though ideal, this is not commonly done in supplement research). However, a decrease in blood pressure is a common occurrence in vasodilation-supporting clinical and recreational supplementation (Khalaf et al., 2019). Therein, at minimum, future research in this topic area should include blood pressure measurements as part of their data collection process. Inclusion of a blood pressure measurement in our data collection process might have helped to indirectly confirm or deny the extent the NOPWS affected vasodilation and our overall findings.

Second, an alarming commonality in nearly all supplements is the standard "scoop" approach to dosage recommendations by manufacturers. For instance, in the current study, no bodyweight recommendation was provided by the manufacturer for supplement serving size (again, this is not uncommon in supplement research). Therein, an individual weighing 45 kg is recommended the same serving size as someone weighing 90 kg. This is concerning from a safety and efficacy standpoint as smaller individuals may take more of the supplement than what is safely recommended, while larger individuals may not be taking enough to realize potential benefits from a supplement. The manufacturer was contacted to determine if dosage should be adjusted based on body mass; however, no change in recommendation was provided. That said, to account for a

potential body mass effect on dosage, the authors performed an ex-post-facto analysis of YMCA bench press performance, relative to body mass. Even when considering the dose of supplement provided per kg of body mass, no statistical difference was observed between the placebo and supplement group. These results cannot fully rule out that body mass should be a consideration for dosage, but the relative amounts of each respective ingredient in this supplement may not have been enough to provide an improvement in performance, regardless of body mass. Future research with all supplements, in addition to those in this study, should examine the effect of bodyweight-dependent dosing on performance outcomes.

Third, the ingredients in this pre-workout supplement do not appear to be synergistic and certainly not more worthwhile than the beneficial effects of supplementation with CM alone (Glenn et al., 2017; Perez-Guisado & Jakeman, 2010; Wax et al., 2016). Thus, those who wish to supplement to support potential NO-induced performance improvements, may wish to consider utilizing one well-researched ingredient (e.g., CM) with mostly positive reviews, rather than a multi-ingredient supplement with less current scientific support.

## CONCLUSION

A new commercially available non-stimulant NOPWS supplement was ineffective at increasing performance in a single set test of muscular endurance. Considering these findings, a lack in performance improvement could be due to a number of factors, including dosage, supplement timing, and number of sets performed in the experiment. Future researchers and recreational users of this type of supplement should consider and be aware of each of these factors in their experimentation methodologies and supplementation regimens, respectively.

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