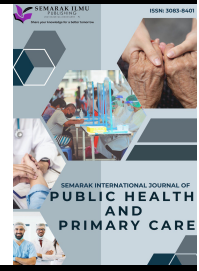




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# Effects of Cocoa Flavanol Supplementation on Maximum Oxygen Uptake in Trained and Non-Trained Male Participants During Intermittent Exercise

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### ABSTRACT

Cocoa flavanols improve vascular function and antioxidant capacity through bioactive compounds such as epicatechin, offering potential ergogenic benefits for aerobic performance. However, fitness status may modulate these effects during intermittent exercise, and the magnitude of this interaction remains unclear. This study examined whether cocoa flavanol supplementation enhances maximum oxygen consumption differently in trained versus non-trained male participants during high-intensity intermittent exercise. A double-blind, randomized, crossover trial enrolled 44 males divided equally into trained and non-trained groups. Participants completed two seven-day supplementation periods: cocoa flavanols (1.35 g daily containing 255 mg epicatechin and 60 mg caffeine) and placebo (3.5 g brown sugar). A 14-day washout separated conditions. The final dose was consumed 1.5 hours before testing. Maximum oxygen consumption was assessed using the Yo-Yo Intermittent Recovery Test Level 1, with performance converted to oxygen uptake values. Dietary intake and physical activity were standardized across trials. Cocoa flavanol supplementation produced significant increases in maximum oxygen consumption in both groups compared to placebo. Trained participants demonstrated a marked improvement (mean difference = 9.818 mL·kg<sup>-1</sup>·min<sup>-1</sup>,  $t(21) = 21.21$ ,  $p < 0.0001$ , partial  $\eta^2 = 0.9554$ ), with high consistency across individuals. Non-trained participants showed smaller but significant gains (mean difference = 4.756 mL·kg<sup>-1</sup>·min<sup>-1</sup>,  $t(21) = 3.378$ ,  $p = 0.0028$ , partial  $\eta^2 = 0.3521$ ), though with greater individual variability. The trained cohort exhibited more than 95% of variance explained by supplementation, whereas the non-trained group demonstrated approximately 35%. Cocoa flavanol supplementation enhances aerobic capacity during intermittent exercise in both trained and non-trained males, with fitness status modulating response magnitude. Trained individuals derive larger, more consistent benefits, likely reflecting superior vascular responsiveness and mitochondrial density. These findings support cocoa flavanols as a viable nutritional strategy for improving intermittent exercise performance, particularly in

#### Keywords:

Cocoa flavanols; high-intensity intermittent exercise; polyphenol supplementation;

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antioxidant effects; exercise recovery;  
fitness status

athletic populations. Individual variability in less-trained groups warrants  
consideration when prescribing supplementation protocols.

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## 1. Introduction

Fruit-derived polyphenols have become an important focus in sports nutrition research due to their potential role in reducing exercise-induced muscle damage and supporting faster recovery [1]. These compounds, found in a wide range of fruits, are known for their antioxidant and anti-inflammatory effects, which may help modulate the body's response to strenuous activity. Evidence suggests that acute intake of fruit-derived polyphenols, usually around 300 mg consumed 1–2 hours before exercise, can improve both endurance and sprint performance by enhancing vascular function and antioxidant defence [2]. Longer-term supplementation, often exceeding 1000 mg per day for several days around intense training, has been shown to lower markers of muscle damage such as creatine kinase and reduce inflammatory responses including C-reactive protein [3]. However, results remain mixed, as some studies have reported contradictory outcomes, including elevated C-reactive protein following bilberry juice intake [4]. These inconsistencies point to the need for further exploration of factors that influence the effectiveness of fruit-derived polyphenols.

Fitness status has emerged as a critical factor that may account for differences in response. Differences in aerobic capacity and training background can shape how individuals respond to supplementation during intermittent exercise. The Yo-Yo Intermittent Recovery Test Level 1 has been widely used to assess aerobic fitness and recovery ability, showing strong links with maximum oxygen uptake. Rampinini *et al.*, [5] reported professional football players typically perform better on the Yo-Yo Intermittent Recovery Test Level 1 compared with amateur players, reflecting their higher aerobic capacity. Training interventions also demonstrate its sensitivity, with recreational players improving by nearly 30% after a 12-week programme [6]. Beyond performance, fitness status also influences physiological responses during high-intensity intermittent activity, with fitter individuals achieving higher external loads and demonstrating greater adaptation [7].

Although many studies have concentrated on short-term supplementation, there is growing attention on the sustained effects of chronic polyphenol intake. Bowtell and Kelly [2] indicate that long-term consumption of polyphenol-rich fruits may be particularly beneficial for less-trained individuals, though the mechanisms behind this remain unclear. This highlights the importance of considering fitness level when evaluating the ergogenic potential of fruit-derived polyphenols.

Despite substantial research on polyphenol supplementation and aerobic performance, a critical gap remains in understanding how fitness status modulates the ergogenic effects of cocoa flavanols during intermittent exercise protocols. While trained athletes may respond differently than non-trained individuals due to differences in vascular function, mitochondrial density, and antioxidant capacity, no studies have systematically compared maximum oxygen consumption responses between these populations following cocoa flavanol supplementation. This knowledge gap limits the development of evidence-based supplementation strategies tailored to different training statuses. Therefore, the current study examines whether fitness status influences maximum oxygen consumption during the Yo-Yo Intermittent Recovery Test Level 1 following cocoa flavanol supplementation. Specifically, this investigation aims to determine whether trained and non-trained male participants demonstrate differential aerobic capacity improvements when supplemented with cocoa flavanols compared to placebo, thereby informing targeted nutritional interventions for athletes and recreational exercisers engaged in intermittent sports.

## 2. Methodology

### 2.1 Research Design

This study employed a double-blind, randomised, cross-over design to investigate whether cocoa flavanol supplementation produced ergogenic effects in trained and non-trained participants. Each participant completed two experimental conditions, cocoa flavanols and placebo, separated by a wash-out phase. The study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Ethics Committee for Human Testing, Universiti Teknologi MARA (REC/02/2024). Written informed consent was obtained from all participants prior to testing. A total of 44 male participants voluntarily enrolled in this study. The trained group ( $n = 22$ ) had a mean age of  $20.2 \pm 1.26$  years, a mean body mass of  $57.3 \pm 5.35$  kg, an average height of  $168 \pm 4.43$  cm, and a mean body mass index of  $20.3 \pm 1.33$  kg/m<sup>2</sup>. The non-trained group ( $n = 22$ ) demonstrated a mean age of  $19.1 \pm 0.47$  years, a mean body mass of  $60.2 \pm 6.79$  kg, a height of  $169 \pm 5.87$  cm, and a mean BMI of  $21 \pm 1.65$  kg/m<sup>2</sup>. The primary outcome of the study was the change in maximum oxygen consumption during intermittent exercise protocols. Performance in the Yo-Yo Intermittent Recovery Test Level 1 was used to quantify aerobic fitness under each supplementation condition. Comparisons were made between trained and non-trained individuals to determine whether fitness status influenced the effect of cocoa flavanol supplementation on maximum oxygen consumption.

### 2.2 Experimental Procedures

Participants attended four laboratory sessions within three weeks. The first visit was used for anthropometric assessment, while the second included familiarisation with the experimental procedures and the Yo-Yo Intermittent Recovery Test Level 1, as well as confirmation of consent. Supplementation commenced on the second visit. The third and fourth visits were experimental trials, performed on day seven of each supplementation period. Participants ingested their final dose of cocoa flavanols or placebo 1.5 hours before completing the Yo-Yo Intermittent Recovery Test Level 1. A minimum 14-day wash-out separated the two conditions, which was deemed sufficient based on the approximately 6-hour half-life of flavanols in humans reported by Muniyappa *et al.*, [8] and to allow recovery from the previous trial. The Yo-Yo Intermittent Recovery Test Level 1 was conducted indoors in a sports hall on a  $2 \times 20$  m running lane marked with cones, following standard protocols described by Gumusdag *et al.*, [9] and Papanikolaou *et al.*, [10]. The test consisted of repeated  $2 \times 20$  m shuttles at progressively increasing speeds, with audio signals dictating the pace. After each run, participants had a 10-second active recovery period within a  $5 \times 2$  m area behind the starting line. The test began with four runs at  $10\text{--}13$  km·h<sup>-1</sup>, followed by seven runs at  $13.5\text{--}14$  km·h<sup>-1</sup>, after which the speed increased in  $0.5$  km·h<sup>-1</sup> increments until exhaustion. Termination criteria included two consecutive failures to reach the line in time or voluntary withdrawal due to fatigue. The total number of levels completed was recorded to determine the distance covered. At the end of the test, participants reported their perceived exertion using the Borg 6–20 scale. Body mass and blood pressure were measured before each trial. Diet and physical activity were standardised before each experimental trial. Participants recorded dietary intake for 36 hours before the first test and replicated it prior to the second test. They were instructed to avoid caffeine and alcohol for 12 and 24 hours, respectively, and to refrain from strenuous activity for 48 hours before each session. Foods rich in polyphenols, such as green tea, grapes, olives, dark chocolate, nuts, and berries, were restricted for 24 hours.

## 2.3 Supplementation Protocol

Two supplementation conditions were tested. The cocoa flavanol capsules provided  $1.35 \text{ g}\cdot\text{day}^{-1}$  of total cocoa flavanols, comprising 255 mg epicatechin, 60 mg caffeine, and approximately 15 kcal, supplied by the Malaysian Cocoa Board. The product was manufactured according to Good Manufacturing Practices and certified Halal by the Department of Islamic Development Malaysia. The placebo consisted of identical capsules containing 3.5 g brown sugar, also providing approximately 15 kcal, produced by Kamron-Production (Selangor, Malaysia). Both preparations were designed for rapid disintegration and absorption in the upper gastrointestinal tract. Coding of supplements and allocation were managed through block randomisation using Research Randomizer®, with blinding maintained until data collection and analyses were complete. Supplementation lasted 7 days, and the final dose was ingested 1.5 hours before testing.

## 3. Results

### 3.1 Anthropometric Measurements

A total of 44 male participants voluntarily enrolled in this study and completed all experimental conditions. Anthropometric characteristics of both groups are presented in Table 1. The trained group ( $n = 22$ ) had a mean age of  $20.2 \pm 1.26$  years, a mean body mass of  $57.3 \pm 5.35$  kg, an average height of  $168 \pm 4.43$  cm, and a mean body mass index of  $20.3 \pm 1.33 \text{ kg/m}^2$ . The non-trained group ( $n = 22$ ) demonstrated a mean age of  $19.1 \pm 0.47$  years, a mean body mass of  $60.2 \pm 6.79$  kg, a height of  $169 \pm 5.87$  cm, and a mean body mass index of  $21 \pm 1.65 \text{ kg/m}^2$ . Both groups completed all supplementation conditions and testing protocols without adverse events.

**Table 1**  
Mean anthropometric measurement

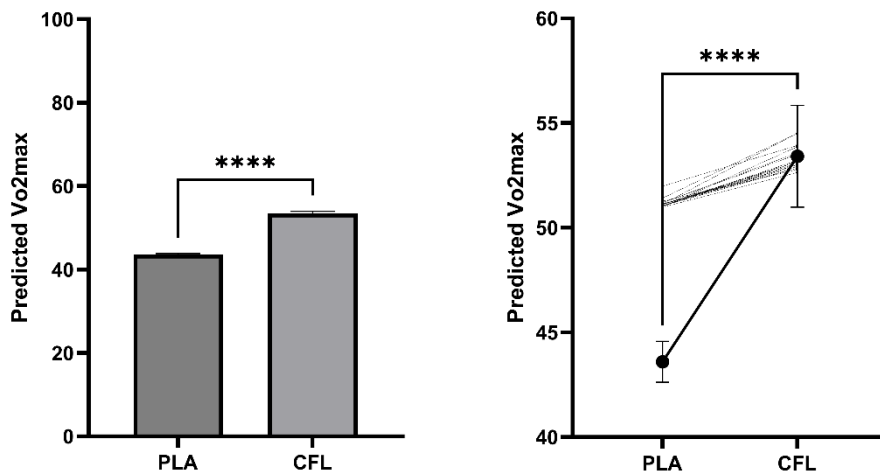
Parameters	Group	
	Trained (n=22)	Non-trained (n=22)
Age (year)	$20.2 \pm 1.26$	$19.1 \pm 0.47$
Body Mass (kg)	$57.3 \pm 5.35$	$60.2 \pm 6.79$
Height (cm)	$168 \pm 4.43$	$169 \pm 5.87$
Body Mass Index ( $\text{kg}\cdot\text{m}^{-2}$ )	$20.3 \pm 1.33$	$21.0 \pm 1.65$

**For example:** This section discusses the results obtained from the surface pressure measurement study. The effects of angle of attack, Reynolds number and leading edge bluntness are discussed in the next sub section.

### 3.2 Changes in Maximum Oxygen Consumption ( $\dot{V}O_2 \text{ max}$ ) in Trained Group

Analysis of the changes in maximum oxygen consumption following supplementation revealed significant differences between the cocoa flavanol and placebo conditions in the trained group. Cocoa flavanol supplementation produced a marked increase in maximum oxygen consumption compared to placebo (Figure 1). A paired t-test confirmed this effect, yielding a highly significant result ( $t(21) = 21.21$ ,  $p < 0.0001$ ). The mean difference between cocoa flavanol and placebo was  $9.818 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  ( $SD = 2.171$ ,  $SEM = 0.4628$ ), with a 95% confidence interval ranging from 8.856 to 10.78. The effect size, as indicated by partial eta squared ( $R^2 = 0.9554$ ), demonstrated that more than 95% of the variance in maximum oxygen consumption could be explained by the supplementation. Additionally, a moderate correlation was observed between paired values ( $r = 0.4496$ ,  $p = 0.0179$ ),

reinforcing the robustness of the paired design and indicating consistent individual responses to cocoa flavanol supplementation within the trained population.

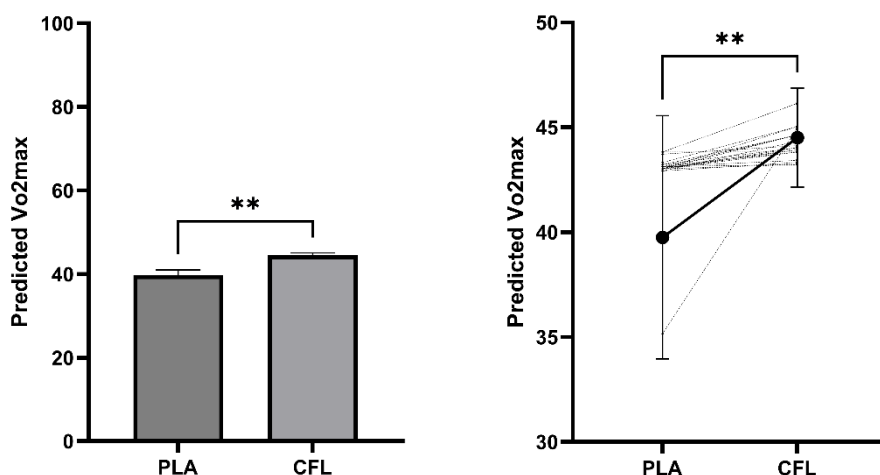


**Fig. 2.** Mean and standard errors of change in maximum oxygen consumption ( $\dot{V}O_2$  max) during YYIRTL1 following PLA and CFL supplementation in trained population

\*Significantly Different ( $p < 0.05$ )

### 3.3 Changes in Maximum Oxygen Consumption ( $\dot{V}O_2$ max) in Non-Trained Group

In the non-trained group, cocoa flavanol supplementation resulted in a significant improvement in maximum oxygen consumption compared to placebo (Figure 2). The paired t-test revealed a significant difference ( $t(21) = 3.378, p = 0.0028$ ). The mean difference was  $4.756 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  ( $SD = 6.604, SEM = 1.408$ ), with a 95% confidence interval ranging from 1.828 to 7.685. The effect size ( $R^2 = 0.3521$ ) indicated that approximately 35% of the variance in maximum oxygen consumption was attributable to supplementation, suggesting a moderate effect. Unlike the trained group, the correlation between paired observations was weak ( $r = -0.1611, p = 0.237$ ), implying that individual variability influenced the outcomes more strongly in this population. Nevertheless, the overall increase in maximum oxygen consumption confirmed that cocoa flavanol supplementation positively affected aerobic capacity in non-trained participants, although the magnitude of improvement was smaller and less consistent than in the trained group.



**Fig. 2.** Mean and standard errors of change in maximum oxygen consumption ( $\dot{V}O_2$  max) during YYIRTL1

following PLA and CFL supplementation in non-trained population

\*Significantly Different ( $p < 0.05$ )

#### 4. Conclusions

Cocoa flavanol supplementation produced meaningful improvements in maximum oxygen uptake relative to placebo in both trained and non-trained participants, with larger and more consistent gains observed in the trained cohort. These findings indicate that cocoa flavanols can enhance aerobic capacity during intermittent exercise, and that fitness status modulates the magnitude of this ergogenic effect. The results are consistent with plausible mechanisms involving improved endothelial function and augmented antioxidant defence, which together may optimise oxygen delivery and utilisation under high-intensity, intermittent demands. Practical application lies in considering cocoa flavanols as an adjunct to training for athletes engaged in intermittent sports, while recognising that responses may vary among less-trained individuals. Caution remains appropriate given the study's male-only sample and the inherent variability within the non-trained group. Further research should establish dose-response relationships, characterise responder phenotypes linked to vascular and redox profiles, include female and broader age groups, and examine transfer to sport-specific performance outcomes and recovery indices over longer time frames.

#### Acknowledgement

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