



## Influence of fitness status on high-intensity intermittent performance in response to fruit-derived polyphenol supplementation

*Influencia del estado físico en el rendimiento intermitente de alta intensidad en respuesta a la suplementación con polifenoles derivados de la fruta*

### Authors

Fatin Nur Shahira Zamri<sup>1</sup>, Nurul Diyana Sanuddin<sup>2</sup>, Adam Linoby<sup>1</sup>, Norlaila Azura Kosni<sup>2</sup>, Nurul Nadiah Shahudin<sup>3</sup>, Mohd Aizzat Adnan<sup>1</sup>

<sup>1</sup>University Technology MARA, Negeri Sembilan Branch, Seremban

<sup>2</sup>University Technology MARA, Pahang Branch, Jengka Campus, Malaysia

<sup>3</sup>University Technology MARA, Shah Alam, Selangor, MALAYSIA

Corresponding author:  
Nurul Diyana Sanuddin  
diyanasanuddin@uitm.edu.my  
Adam Linoby  
linoby@uitm.edu.my

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### Abstract

**Objective:** This study examined the effects of cocoa flavanol (CFL) supplementation on high-intensity intermittent performance and aerobic capacity, focusing on the role of fitness status. **Methodology:** Forty-four male participants, divided into trained (n = 22) and non-trained (n = 22) groups, completed the Yo-Yo Intermittent Recovery Test Level 1 (YYIRTLL1) after seven days of CFL or placebo supplementation. The study employed a double-blind, randomized cross-over design.

**Results:** Results showed that CFL supplementation significantly improved performance in both groups, with the trained group demonstrating a greater increase in total distance covered during YYIRTLL1 compared to placebo (mean difference: 1169 m,  $P < 0.01$ ). Additionally, VO<sub>2</sub>max significantly improved in the trained group (mean difference: 9.82 mL·kg<sup>-1</sup>·min<sup>-1</sup>,  $P < 0.01$ ). In the non-trained group, CFL supplementation also led to significant enhancements in YYIRTLL1 performance (mean difference: 438.2 m,  $P < 0.01$ ) and VO<sub>2</sub>max (mean difference: 4.76 mL·kg<sup>-1</sup>·min<sup>-1</sup>,  $P < 0.01$ ), although the improvements were less pronounced than in the trained group. These findings indicate that CFL supplementation effectively boosts performance and aerobic capacity, with more substantial benefits observed in trained individuals. Further studies are needed to investigate long-term effects and the mechanisms underlying differential responses based on fitness level.

### Keywords

Cocoa flavanols, high-intensity intermittent exercise, polyphenol supplementation, antioxidant effects, exercise recovery, fitness status.

### Resumen

**Objetivo:** Este estudio examinó los efectos de la suplementación con flavanoles de cacao (CFL) en el rendimiento de alta intensidad intermitente y la capacidad aeróbica, enfocándose en el papel del nivel de condición física. **Metodología:** Cuarenta y cuatro participantes masculinos, divididos en grupos entrenados (n = 22) y no entrenados (n = 22), realizaron la prueba de recuperación intermitente Yo-Yo Nivel 1 (YYIRTLL1) después de siete días de suplementación con CFL o placebo. El estudio empleó un diseño cruzado, aleatorizado y doble ciego.

**Resultados:** Los resultados mostraron que la suplementación con CFL mejoró significativamente el rendimiento en ambos grupos, con el grupo entrenado demostrando un mayor aumento en la distancia total recorrida durante el YYIRTLL1 en comparación con el placebo (diferencia media: 1169 m,  $P < 0.01$ ). Además, el VO<sub>2</sub>max mejoró significativamente en el grupo entrenado (diferencia media: 9.82 mL·kg<sup>-1</sup>·min<sup>-1</sup>,  $P < 0.01$ ). En el grupo no entrenado, la suplementación con CFL también condujo a mejoras significativas en el rendimiento del YYIRTLL1 (diferencia media: 438.2 m,  $P < 0.01$ ) y en el VO<sub>2</sub>max (diferencia media: 4.76 mL·kg<sup>-1</sup>·min<sup>-1</sup>,  $P < 0.01$ ), aunque las mejoras fueron menos pronunciadas que en el grupo entrenado. Estos hallazgos indican que la suplementación con CFL aumenta eficazmente el rendimiento y la capacidad aeróbica, con beneficios más sustanciales observados en individuos entrenados. Se necesitan más estudios para investigar los efectos a largo plazo y los mecanismos subyacentes a las respuestas diferenciales según el nivel de condición física.

### Palabras clave

Flavanoles de cacao, ejercicio intermitente de alta intensidad, suplementación con polifenoles, efectos antioxidantes, recuperación del ejercicio, nivel de condición física.

## Introduction

In recent years, fruit-derived polyphenols (FDPs) have gained considerable attention for their potential to mitigate exercise-induced muscle damage (EIMD) and enhance post-exercise recovery. Polyphenols, as bioactive compounds found in many fruits, possess antioxidant and anti-inflammatory properties that can influence the physiological responses to exercise. Acute supplementation of FDPs, typically around 300 mg administered 1–2 hours before exercise, has been shown to improve endurance and sprint performance, likely due to enhanced antioxidant and vascular functions (Bowtell & Kelly, 2019). Chronic doses, exceeding 1000 mg·day<sup>-1</sup>, administered over at least three days before and after intense physical activity, significantly reduce markers of muscle damage, including creatine kinase, and inflammatory markers such as C-reactive protein (CRP) (Doma et al., 2020). However, some studies have reported inconsistencies in the effects of FDP supplementation, with a subset of trials indicating increased inflammation in certain conditions, such as the potential rise in CRP levels following bilberry juice supplementation (Lynn et al., 2018). Despite these mixed outcomes, the potential of FDPs to improve recovery remains evident, though further investigation is warranted to understand the variability in response, especially concerning individual fitness levels. The influence of fitness status on exercise performance, particularly in intermittent performance tests, is increasingly being studied in relation to FDP supplementation. The Yo-Yo Intermittent Recovery Test (YYIRT1), a widely used measure of aerobic capacity and recovery, has been employed to assess fitness status across various athletic populations. Research comparing professional and amateur soccer players highlighted a strong correlation ( $r = 0.74$ ) between YYIRT1 performance and maximum oxygen uptake ( $\dot{V}O_{2\max}$ ), suggesting a higher aerobic capacity in professional athletes (Rampinini et al., 2009). A 12-week training program for recreational football players further demonstrated a substantial 30% improvement in YYIRT1 performance, emphasizing the test's sensitivity to fitness changes (Póvoas et al., 2019). Moreover, fitness status has also been linked to variations in immune responses during intermittent exercise. High-fitness groups exhibited significantly higher external training loads, recording  $34 \pm 13$  accelerations compared to  $17 \pm 7$  in lower-fitness individuals, underscoring the impact of fitness on both performance and physiological responses (Valvassori et al., 2020). These findings illustrate that fitness status plays a critical role in modulating the effects of FDP supplementation during intermittent performance.

Furthermore, while much of the research has focused on the acute effects of FDP supplementation, there is growing interest in understanding the long-term impacts of chronic polyphenol consumption, particularly in athletes with varying levels of fitness. Studies have shown that long-term supplementation with polyphenol-rich fruits can reduce markers of muscle damage and improve recovery over extended periods, with some evidence suggesting that these benefits may be more pronounced in less-trained individuals (Bowtell & Kelly, 2019). However, the mechanisms by which fitness status influences the long-term efficacy of FDP supplementation remain poorly understood. This underscores the importance of conducting longitudinal studies to explore the sustained effects of polyphenols on recovery, especially in athletes undergoing varying training intensities. Given the variability in response to FDP supplementation based on fitness levels, it is essential to investigate whether the ergogenic effects of FDPs differ between trained and untrained individuals during high-intensity intermittent performance tests. This study aims to address this gap by exploring how fitness status influences the impact of FDP supplementation on performance outcomes during the YYIRT1 and other relevant intermittent exercise protocols. Through a comparative analysis of trained and untrained participants, the study will provide insights into whether fitness level modulates the recovery-enhancing properties of FDP supplementation, offering practical implications for optimizing supplementation strategies in athletic populations.

## Method

### Study Design

This double-blind, randomized, cross-over study was designed to investigate whether fruit-derived polyphenol (FDP) supplementation elicited potential ergogenic effects between trained and non-trained participants. On the first visit, subjects reported to the exercise lab for anthropometric collection. During the second visit, prior to the first experimental trial, all subjects were familiarized with the experimental protocol, YYIRT1 and signed consent forms before testing. Subjects were required to visit the lab on



four occasions, spread over a three-week period, with 7 days in between. Subjects received their supplementation on the second visit. The experimental trial days (visits three and four) that followed were each on day 7 of the cocoa flavanols (CF) or placebo (PLA) supplementation period, with the last supplemental bolus being ingested 1.5 hours prior to performing the YYIRTL1. There was a gap of at least 14 days as a wash-out period between trials according to the half-life of flavanols, the main active compound of CFL in humans after oral administration being approximately ~6 hours. Therefore, a 14-day washout period was deemed adequate to eliminate the administered dose and to allow recovery from the exercise intervention (Muniyappa et al., 2008). The study protocol adhered to the principles outlined in the Declaration of Helsinki and received approval from the Ethics Committee for Human Testing at Universiti Teknologi MARA (Code: REC/02/2024).

### Participants

A total of 44 male participants were recruited on a voluntary basis to participate in this study. The trained group had an average age of  $20.2 \pm 1.26$  years, a body mass of  $57.3 \pm 5.35$  kg, a body height of  $168 \pm 4.43$  cm, and a body mass index (BMI) of  $20.3 \pm 1.33$  kg/m<sup>2</sup>, meanwhile the non-trained group had an average age of  $19.1 \pm 0.47$  years, a body mass of  $60.2 \pm 6.79$  kg, a body height of  $169 \pm 5.87$  cm, and a body mass index (BMI) of  $21 \pm 1.65$  kg/m<sup>2</sup>.

Table 1 showed the physical characteristics of participants involved in this study.

Table 1. Physical Characteristics of Participants

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 (kg·m <sup>-2</sup> )	$20.3 \pm 1.33$	$21 \pm 1.65$

### Experimental Protocols

The warm-up and the YYIRTL1 were performed indoors in a sports hall, on a 2 by 20 m running lane that was marked by cones, as described previously by Gumusdag et al. (2013) and Papanikolaou et al. (2019). The test consisted of repeated 2 x 20 m sprints between a starting, turning, and finishing line at a progressively increasing speed controlled by audio beeps from an audio system. Between each 2 x 20 m run, subjects had a 10-s active recovery period in an area of 5 x 2 m that was marked by cones behind the start/finishing line. YYIRTL1 had 4 running bouts at 10–13 km·h<sup>-1</sup> and another 7 runs at 13.5–14 km·h<sup>-1</sup>, after which it continued with stepwise 0.5 km·h<sup>-1</sup> speed increments after every stage until exhaustion. YYIRTL1 usually took 6 to 20 minutes. The test was ended if the player failed to reach the finish line within the given time frame on two consecutive occasions or if the player felt unable to continue (volitional exhaustion). The total number of levels was recorded and used to determine the total distance covered (m) during the test. Immediately after completing the YYIRTL1, subjects rated their perceived exertion on a Borg 6-20 scale.

#### Supplementation Protocol and Standardization of Physical Activity and Diet

Subjects underwent 2 interventional trials where they consumed CFL or a PLA. Subjects recorded their dietary intake 36 hours prior to the first test day (visit 3). They were subsequently instructed to replicate their dietary intake 36 hours prior to the second test day (visit 4). Subjects were asked to avoid caffeine and alcohol for 12 hours and 24 hours prior to each test day, respectively. They were also asked not to perform heavy exercise in the last 48 hours and to abstain from foods with a high polyphenol content (green tea, grapes, olives, dark chocolate, hazel and pecan nuts, berries) for the last 24 hours. Subjects were weighed, and blood pressure was taken.

#### Supplementation Preparation

The supplementation protocol for this study included two conditions: CFL and a placebo (PLA). The CFL supplement provided 1.35 g/day of total CFL, comprising 255 mg of epicatechin, 60 mg of caffeine, and 15 kcal, and was obtained from Malaysian Cocoa Board (MCB), and was produced in accordance with

Good Manufacturing Practices (GMP) guidelines, certified Halal by the Department of Islamic Development Malaysia (JAKIM). The PLA, produced by Kamron-Production in Selangor, Malaysia, consisted of identical non-transparent capsules filled with 3.5 g of brown sugar, also delivering approximately 15 kcal. Both supplements were designed to disintegrate and dissolve rapidly in the upper gastrointestinal tract. The supplement packages were coded and block randomization performed by using the online tool Research Randomizer® to assign participants to either the CFL or PLA group. The blinding was maintained until data collection was complete and all analyses had been finalized.

## Measurements

### High Intensity Intermittent Exercise Test

A modified protocol of YYIRTL1 was adopted from Thompson et al. (2016). Participants performed the YYIRTL1 test until volitional exhaustion or when they were 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 each participant and estimated the participants' maximal aerobic capacity using the mathematical equation by Bangsbo et al. (2008).

$$\text{Predicted } \dot{V}O_{2\max} (\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \text{YYIRTL1 distance (m)} \times 0.0084 + 36.4$$

## Statistical Analysis

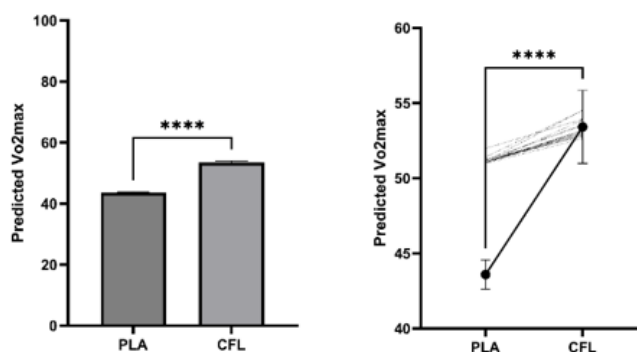
Data were presented as means and standard deviations. The assumption of normality was assessed using the Shapiro-Wilk test. A paired t-test was employed to evaluate the mean difference between the two conditions (CFL & PLA). When a significant effect was observed, pairwise comparisons were conducted using the Bonferroni post-hoc test. Statistical significance was established at a threshold of  $p < 0.05$ .

## Results

### Changes in YYIRTL1 Distance

The paired t-test comparing YYIRTL1 total distance between CFL and PLA conditions in the trained group revealed a highly significant difference ( $P < 0.0001$ ), confirming strong statistical significance at the  $P < 0.05$  level. The t-value was 21.23 with 21 degrees of freedom, and the mean difference between conditions was 1169 m (SD = 258.3, SEM = 55.08). The 95% confidence interval ranged from 1055 to 1284, indicating precise estimation. The R squared value of 0.9555 shows high variance explained by the treatment. Pairing effectiveness was significant ( $r = 0.4499$ , one-tailed  $P = 0.0178$ ), demonstrating a substantial performance improvement with CFL.

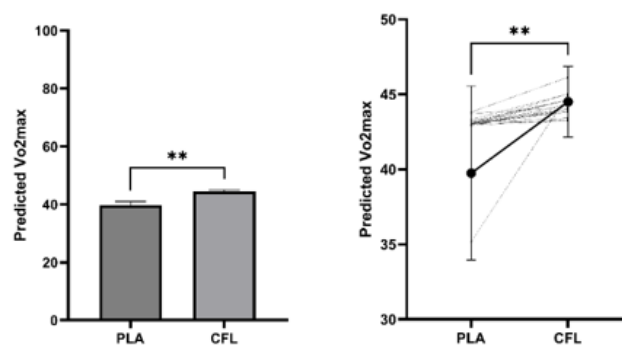
Figure 1. Mean and Standard Errors of Change in Total Distance during YYIRTL1 following PLA and CFL supplementation in Trained Population.



\*Significantly Different ( $p < 0.05$ )

The paired t-test comparing YYIRTL1 total distance between CFL and PLA conditions in the non-trained group revealed a highly significant difference ( $P < 0.0001$ ), indicating strong statistical significance at the  $P < 0.05$  level. The t-value was 8.188 with 21 degrees of freedom, and the mean difference between conditions was 438.2 m (SD = 251, SEM = 53.52). The 95% confidence interval ranged from 326.9 to 549.5, reflecting reasonable precision. The R squared value of 0.7615 shows a substantial variance explained by the treatment. Pairing effectiveness was significant ( $r = 0.4777$ , one-tailed  $P = 0.0123$ ), demonstrating improved performance with CFL.

Figure 2. Mean and Standard Errors of Change in Total Distance during YYIRTL1 following PLA and CFL supplementation in Non-trained Population.



\*Significantly Different ( $p < 0.05$ )

## Discussion

The results of the current study highlight the effects of CFL supplementation on intermittent performance in trained individuals, particularly in the context of YYIRTL1. In the trained group, the significant increase in total distance covered following CFL ingestion, as compared to PLA, supports the notion that polyphenol supplementation may enhance performance outcomes during intermittent, high-intensity activities. This is consistent with prior studies in trained athletes, which demonstrated that CFL supplementation improved aerobic capacity and muscle recovery. For instance, a study on elite rugby players reported an improvement of 97 meters in YYIRTL1 performance following CFL supplementation, although the result did not reach statistical significance ( $P = 0.57$ ) (de Carvalho et al., 2019). In contrast, our findings suggest a more substantial improvement in trained athletes, with a greater magnitude of difference. These discrepancies could be attributed to differences in supplementation duration, dosage, or variations in the athletes' fitness status. Studies also suggest that polyphenol supplementation may not uniformly enhance intermittent performance, particularly under conditions of exhaustive high-intensity exercise, where selective effects on aerobic performance markers such as prefrontal oxygenation have been noted without direct improvements in performance metrics (Decroix et al., 2018). This variability underscores the need for further research to understand the mechanisms by which CFL influences intermittent exercise in different athletic populations.

In contrast to the trained cohort, the effects of CFL supplementation on intermittent performance in non-trained individuals were less pronounced in the current study. The non-trained participants displayed no significant improvement in YYIRTL1 distance following CFL ingestion, a finding consistent with previous literature. For example, a study involving collegiate rugby players found that while the CFL group ran 97 meters further than the PLA group during the YYIRTL1, the result was not statistically significant ( $P = 0.57$ ) (de Carvalho et al., 2019). Additionally, research in non-trained individuals has suggested that CFL's benefits may be more evident in metabolic recovery, such as improvements in glucose metabolism and reduced muscle fatigue, rather than direct enhancements in exercise performance (Melo et al., 2021). The lack of performance improvement in our study may be related to baseline fitness levels, as individuals with lower fitness tend to exhibit less pronounced performance gains from polyphenol supplementation, likely due to diminished oxidative stress and recovery capacity. This highlights the complexity of CFL supplementation in non-trained populations and suggests that while there may be metabolic benefits, performance-related outcomes may remain limited.

The current study also explored the effects of CFL supplementation on VO<sub>2</sub>max, particularly in trained athletes. Our findings align with the broader literature, which presents mixed results regarding the impact of polyphenols on maximal oxygen uptake. While the trained participants in our study did not exhibit significant improvements in VO<sub>2</sub>max following CFL ingestion, previous studies have reported similar outcomes. A study on well-trained athletes noted that despite increased antioxidative capacity and nitric oxide production, VO<sub>2</sub>max and exercise performance did not significantly improve after acute CFL supplementation (Decroix et al., 2018). In contrast, other research involving healthy elderly participants showed a significant improvement in peak VO<sub>2</sub>max by 2.5 ml/min/kg ( $P < 0.01$ ) after 30 days of daily CFL supplementation (Gröne et al., 2023). These discrepancies suggest that while CFL may influence physiological markers of recovery and oxygenation, its effects on maximal aerobic performance are variable and may depend on factors such as supplementation duration, dosage, and the athlete's baseline fitness. In non-trained individuals, the effects of CFL supplementation on VO<sub>2</sub>max were similarly modest. Our findings, which showed no significant changes in VO<sub>2</sub>max following supplementation, are consistent with previous studies. For example, a randomized controlled trial in sedentary middle-aged adults demonstrated a 15% decrease in the time constant of VO<sub>2</sub> kinetics during moderate-intensity exercise ( $P = 0.019$ ), but no significant improvements in VO<sub>2</sub>max during severe-intensity exercise (Sadtler et al., 2021). Furthermore, another study involving non-trained individuals consuming cocoa products found no significant improvement in exercise tolerance or VO<sub>2</sub>max, despite reductions in oxidative stress markers (Corr et al., 2020). These results emphasize the variability in responses to polyphenol supplementation and suggest that baseline fitness levels play a critical role in determining the effectiveness of CFL on maximal oxygen consumption and aerobic capacity.

The variability in response to CFL supplementation across different fitness levels observed in this study mirrors findings from other research on polyphenols and exercise performance. Genetic predisposition, fitness status, and exercise type have all been identified as key factors contributing to inter-individual variability in response to polyphenol supplementation. For example, a study on grape pomace supplementation reported that variations in gut microbiota composition and miRNA expression explained differences in insulin response among individuals, with responders exhibiting lower levels of Prevotella and Firmicutes ( $P < 0.05$ ) (Ramos-Romero et al., 2020). Similarly, research has shown that genetic factors play a substantial role in determining cardiorespiratory fitness adaptations to training, with twin studies highlighting a strong genetic component (Ross et al., 2019). These findings underscore the importance of considering genetic, physiological, and microbiological factors when assessing the effectiveness of polyphenol supplementation, particularly in diverse populations with varying fitness levels.

## Conclusions

This study demonstrates that CFL supplementation significantly enhances high-intensity intermittent performance and maximal oxygen uptake, particularly in trained individuals. The findings support the potential of polyphenol-rich supplements as an effective intervention to improve aerobic capacity and performance during intermittent exercises like the YYIRTL1. Although non-trained participants also saw significant improvements, the effects were less pronounced. These findings highlight the potential of polyphenol-rich supplements to enhance aerobic capacity and recovery, but the benefits may vary based on fitness level. Future research should explore long-term effects, genetic factors influencing responses, and sex differences to better understand CFL's impact across diverse populations.

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### Authors and translators' details:

Fatin Nur Shahira Zamri  
Nurul Diyana Sanuddin  
Adam Linoby  
Norlaila Azura Kosni  
Nurul Nadiah Shahudin  
Mohd Aizzat Adnan

fatinnurshahira@uitm.edu.my  
diyanasanuddin@uitm.edu.my  
linoby@uitm.edu.my  
norlailaazura@uitm.edu.my  
nadiyah\_shahudin@uitm.edu.my  
mohda5782@uitm.edu.my

Main Author  
Corresponding Author  
Corresponding Author  
Author  
Author  
Author

