

Elucidating the Effects of Single and Combine Fruit-Dervied Polyphenol Supplementation on High-Intensity Intermittent Performance

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ABSTRACT

A double-blind, randomized, placebo-controlled study aimed to investigate the effects of cocoa flavanols (CFL), berry polyphenols (BRS), their combination (CFL+BRS), and a placebo (PLA) on time to exhaustion during exercise. A total of 52 male participants were randomly allocated in four different groups (CFL+BRS, CFL, BRS, PLA), underwent one interventional trial where they consumed different intervention allocated for 7 days. The participant performed the cycling exercise test (Ramp Incremental Test and Time to Exhaustion test) on an electronically braked cycle ergometer. The time to exhaustion was recorded as the duration from the beginning of the incremental test to the point of task failure. A two-way ANOVA revealed a significant difference between the four conditions ($p = 0.0083$), with the CFL+BRS group demonstrating the highest mean performance (598.8 ± 148.6), followed by the CFL group (563.1 ± 199.0). The BRS group (452.7 ± 127.6) had the lowest mean performance, slightly below the PLA group (480.6 ± 119.9). The superior performance of the CFL+BRS group highlights the synergistic benefits of mixed polyphenol supplementation, combining enhanced vascular function, antioxidant defense, and anti-inflammatory effects. These findings suggest that mixed-source polyphenol supplementation is more effective than single sources in improving endurance and recovery. The study underscores the potential of dietary diversity and targeted supplementation for optimizing athletic performance and highlights the need for further research into the mechanisms and long-term effects of polyphenol combinations.

Keywords: Cocoa flavanols, time to exhaustion, polyphenol supplementation, antioxidant effects, exercise recovery, fitness status

INTRODUCTION

Polyphenols are a class of naturally occurring compounds found in plants and distinguished by the presence of at least one phenol unit per molecule. Various classes of polyphenols can be distinguished. Included in this category are flavonoids, lignans, phenolic acids, and stilbenes. Recent evidence highlights that categorizing polyphenol as flavonoids or non-flavonoids is advantageous [1]. Ingestion of a single polyphenol can give rise to many different metabolites, which was clearly shown in an elegant investigation where participants consumed 500 mg of ¹³C labelled cyanidin-3-glycoside, and 17 different labelled metabolites were detected in plasma over the subsequent 48 h (de Ferrars et al., 2014). Fruits and vegetables and fruit-derived polyphenol supplements contain a blend of polyphenols, and thus the pharmacokinetics and metabolism after ingestion of whole foods or fruit-derived supplements are even more complex. There is also a high degree of inter-individual variation in bioavailability partly dictated by differences in the gut microbiome [6].

Several attempts have been made to investigate the efficacy of fruit-derived polyphenol intermittent exercise, but the results have been contradictory. Previous research reported that there is no improvement of single FDP supplementation effect were observed on functional performance, inflammatory markers or oxidative stress after water-polo stimulation [1]. In contrast, in an investigation towards semi-professional soccer players following the Loughborough Intermittent Shuttle Test (LIST), the findings reported beneficial effects of single FDP ingestion [9]. The literature presented an improvement recovery of maximum voluntary isometric contraction (MVIC), counter movement jump (CMJ), and agility along with lower muscle soreness (DOMS) and IL-6 concentrations when single source of FDP was administered to athletes against a placebo. However, it is important to note that athletes were instructed to consume a diet low in phenolic compounds commencing 48 hours before supplementation. It is uncertain whether the same advantages would result from consuming independent or mixed in addition to an athlete's usual diet, which may contain sufficient antioxidant and anti-inflammatory elements.

Research on FDP supplementation has evaluated its ability in aiding recovery, but research findings are unclear. In brief, even though scientific evidence shows that FDP supplementation is an effective supplement, it cannot be ignored that it should be expanded on a consistent scientific basis. Based on previous studies, the ergogenic effects of FDP administration remain controversial. While the studies of FDP are evaluated in terms of its ability in promoting recovery, some studies show that it has no effect and some studies report its positive effect [1]. As more research on FDP supplementation continues to be published, there remains confusion about FDP's potential to aiding recovery. Recent research on the effects of independent or combination fruit-derived polyphenol supplementation in enhancing recovery and performance during intermittent activity is limited, and due to dietary restrictions. Therefore, the purpose of this study is to examine the efficacy of single or mixed fruit-derived polyphenol supplementation in promoting recovery after intermittent exercise.

METHODOLOGY

Study Design

This double-blind, randomized, placebo-controlled study was designed to investigate the effect of single or combined fruit-derived polyphenol supplementation on high-intensity intermittent exercise. On the first visit, subjects reported to the exercise laboratory for anthropometric collection. During the second visit, the participants undergo cycling exercise test to identify the power output at gas exchange threshold (GET) and power at maximal aerobic effort ($\dot{V}O_{2\max}$) in order to calculate the constant power output. Then, during the third visit, prior to the experimental trials, all subjects were familiarized with the experimental protocol and signed consent form before testing. Subjects were required to visit the laboratory on four occasions, spread over a four-week period, with 7 days in between. Subjects received their supplementation on the third visit. The experimental trial days (visit four) that followed were each on day 7 of the combination of cocoa flavanols and berries (CFL+BRS), cocoa flavanols (CFL), berries and cherries (BRS) or a placebo (PLA) supplementation period, with the last supplemental bolus being ingested 1.5 hours prior to performing the YYIRTL1. The study protocol adhered to the principles outlined in the Declaration of Helsinki [13] and received approval from the Ethics Committee for Human Testing at Universiti Teknologi MARA (Code: REC/02/2024)(PG/FB/9).

Participants

A total of 52 male participants were recruited on a voluntary basis to participate in this study. The participants been divided into four groups, consisting of 13 participants per group. The combination of cocoa flavanols and berries (CFL+BRS) group had an average age of 19.08 ± 1.04 years, a body mass of 63.37 ± 11.23 kg, a body height of 1.72 ± 0.05 m and a body mass index (BMI) of 21.61 ± 3.10 g/m². Meanwhile, the cocoa flavanols (CFL) group had an average age of 19.92 ± 0.64 years. Their body mass was 68.98 ± 9.19 kg, with a height of 1.70 ± 0.05 meters, and a BMI of 23.92 ± 3.23 kg/m². The berries and cherries group (BRS) had an average age of 19.69 ± 0.63 years, body mass of 63.29 ± 9.78 kg with an average height of 1.68 ± 0.05 meters. Their BMI was 22.53 ± 3.59 kg/m². The placebo group had an average age of 19.15 ± 1.14 years, body mass at 81.51 ± 24.90 kg, average height of 1.75 ± 0.07 meters and their BMI, 26.47 ± 7.61 kg/m².

Experimental Protocols

Cycle Ergometry

The participants performed the cycling exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Ramp incremental tests to volitional exhaustion (30W/min) were performed to identify the power output at gas exchange threshold (GET) and power at maximal aerobic effort ($\dot{V}O_{2\max}$), while recording the heart rate continuously. The highest 30-second average HR was used as a surrogate for the corresponding power output [7]. The constant power output for cycling time trials test been calculated as below:

Severe-intensity power output = Power at GET + 0.7 x (Power at maximal aerobic effort – Power at GET)

Ramp Incremental Test

Ramp incremental tests were employed by having a 3 min baseline period where subjects pedaled at 30W at a self-selected cadence between 75 and 90 rpm. Following this, the work rate linearly increased at a rate of $30W \cdot min^{-1}$ until task failure. Task failure occurred when the pedal rate fell by more than 10 rpm below the target cadence [12].

“Time to exhaustion” test

The baseline cycling repeated during the beginning of time to exhaustion test. After the baseline period, the workload abruptly increased to approximately 70% of the participant's power output. The participants were instructed to maintain a consistent cadence throughout the test. The test is terminated when the participant reaches the limit of their exercise tolerance. The point of exhaustion is defined as the moment when the participant can no longer maintain the required cadence (a drop of more than 10 rpm from their chosen cadence) for more than 10 seconds, even with verbal encouragement. This is considered the limit of exercise tolerance, indicating that the participant has reached their maximum physical effort. The self-selected cadence, seat height and handlebar configuration were recorded and reproduced on subsequent visits.

Supplementation Protocol and Standardization of Physical Activity and Diet

Subjects underwent one interventional trial where they consumed either combination of cocoa flavanols and berries (CFL+BRS), cocoa flavanols (CFL), berries and cherries (BRS) or a placebo (PLA) for 7 days. Subjects recorded their dietary intake 36 hours prior to the test days (visit 4 and visit 5). 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 four conditions: combination of cocoa flavanols and berries (CFL+BRS), cocoa flavanols (CFL), berries (BRS) and a placebo (PLA). The CFL supplement provided 1.35 g/day of total cocoa flavanols, comprising 255 mg of epicatechin, 60 mg of caffeine, and 15 kcal, and was obtained from Malaysian Cocoa Board (MCB). Meanwhile, the berries and cherries ingested were acquired from CK Ingredient Sdn. Bhd. Both supplements was produced in accordance with Good Manufacturing Practices (GMP) guidelines, certified Halal by the Department of Islamic Development Malaysia (JAKIM). The placebo, 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. All the 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

Time to Exhaustion

The “time to exhaustion” was recorded as the duration from the beginning of the incremental test to the point of task failure [12].

Statistical Analysis

Data were presented as means and standard deviations. The assumption of normality was assessed using the Shapiro–Wilk test. A two-way ANOVA was employed to evaluate the mean difference between the four conditions (CFL+BRS, CFL, BRS & PLA). GraphPad Prism software (version 9.0, GraphPad Software Inc., La Jolla, California, USA) was used for the data analysis, with statistical significance was established at a threshold of $p < 0.05$.

RESULTS

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

Table 1 Physical Characteristics Of Participants

Participants	Group			
	CF BR (n=13)	CF (n=13)	BR (n=13)	PLA (n=22)
Age (year)	19.08 ± 1.04	19.92 ± 0.64	19.69 ± 0.63	19.15 ± 1.14
Body Mass (kg)	63.37 ± 11.23	68.98 ± 9.19	63.29 ± 9.78	81.51 ± 24.90
Height (m)	1.72 ± 0.05	1.70 ± 0.05	1.68 ± 0.05	1.75 ± 0.07
Body Mass Index ($\text{kg}\cdot\text{m}^{-2}$)	21.61 ± 3.10	23.92 ± 3.23	22.53 ± 3.59	26.47 ± 7.61

Changes in “Time to Exhaustion”

The group mean changed in time to exhaustion following different ingestion, combination of cocoa flavanols and berries (CFL+BRS), cocoa flavanols (CFL), berries (BRS) and a placebo (PLA), as shown in Figure 1 and 2. The results of the two-way ANOVA conducted on the completion time of “time to exhaustion” comparing four different conditions indicate a statistically significant difference between the four conditions ($p = 0.0083$), as the p-value is below the significance level. The CFL+BRS group demonstrated the highest mean performance (598.8 ± 148.6), highlighting its superior effectiveness compared to the other conditions, followed by the CF groups (563.1 ± 199.0). In contrast, the BRS group had the lowest mean performance (452.7 ± 127.6) compared to PLA group (480.6 ± 119.9) that slightly outperformed the BRS group. These findings suggest that the CFL+BRS group is the most effective intervention compared to other conditions in enhancing performance as measured by the time to exhaustion.

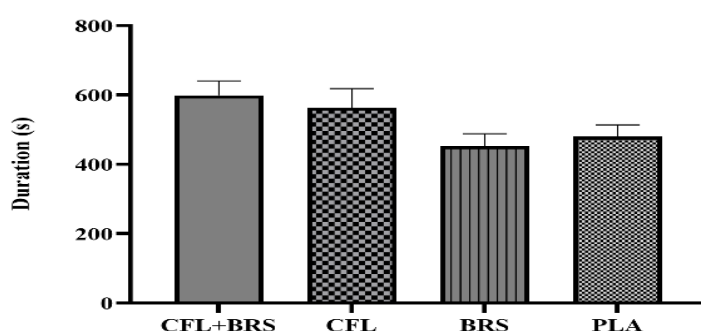


Fig.1 Mean and Standard Errors in Time to Exhaustion (s) following four supplementations

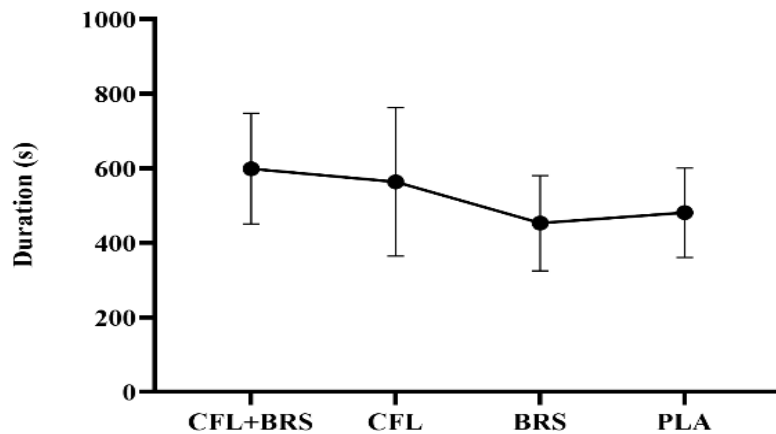


Fig.2 Mean and Standard Errors in Time to Exhaustion (s) following four supplementations

DISCUSSION

The result of current study demonstrated the effect of different ingestion such as combination of cocoa flavanols and berries (CFL+BRS), cocoa flavanols (CFL), berries (BRS) and a placebo (PLA) in improving time to exhaustion during exercise. The findings indicate that the cocoa flavanols (CFL) group and berries and cherries (BRS) is the most effective intervention, outperforming the effects of either single source of polyphenol or the placebo condition. The statistical analysis reported a significant difference between the four experimental conditions ($p = 0.0083$), in which the CFL+BRS showed the highest mean time to exhaustion followed with the other groups. These results strongly support the hypothesis that a mixed polyphenol supplementation strategy, combining the cocoa flavanols and berries provides a synergistic effect that provides performance benefits compared to either source alone [3]. The superior performance of CFL+BRS group can be explained by the complementary mechanism of actions offered by cocoa flavanols and berry polyphenol. The cocoa flavanols are well-documented for the ability to enhance the endothelial function by stimulating the nitric oxide (NO) production [Jaramillo]. This vasodilatory effect improves the blood flow, ensuring an efficient oxygen and nutrients delivery to active muscles during exercise, which is important for delaying the fatigue [4]. In addition, cocoa flavanols exert powerful antioxidants effects by scavenging reactive oxygen species (ROS) that accumulate during high-intensity exercise, thereby reducing oxidative damage to muscle cells and preserving their function. The flavanols' anti-inflammatory properties further support recovery by downregulating pro-inflammatory cytokines such as IL-6 and TNF- α , which are typically elevated following intense physical activity [1].

On the other hand, berry polyphenols, particularly anthocyanins and ellagitannins, contribute to performance improvements through distinct yet complementary pathways. These polyphenols enhance intracellular antioxidant defense systems, neutralizing ROS at the cellular level and preventing oxidative damage to critical structures such as mitochondria, lipids, and proteins [9]. Furthermore, berry polyphenols are known to support mitochondrial function and biogenesis, optimizing energy production during prolonged exercise [9]. Like cocoa flavanols, berry polyphenols exhibit anti-inflammatory effects by stabilizing immune responses and reducing neutrophil-driven oxidative bursts, which can exacerbate muscle damage [11]. Together, these mechanisms ensure that berry polyphenols protect against both the metabolic and mechanical stresses associated with exercise. The synergy observed in the CFL+BRS group arises from the integration of these complementary effects. Therefore, by combining the vascular and antioxidant benefits of cocoa flavanols with the intracellular ROS scavenging and mitochondrial support provided by berry polyphenols, the CFL+BRS supplementation delivers a broader spectrum of protective effects than either source alone [1]. This mixed-source approach not only enhances oxygen delivery and utilization during exercise but also mitigates muscle damage and supports faster recovery. Importantly, the combination appears to reduce variability in individual response, which is often influenced by factors such as gut microbiome composition and dietary habits, by providing a diverse range of bioactive compounds [9].

In contrast, the CFL group, while effective, showed slightly lower mean performance than the CFL+BRS

group. This difference highlights the limitations of relying solely on vascular and antioxidant benefits without the intracellular and mitochondrial support offered by berries. The BRS group, which underperformed relative to both the CFL and CFL+BRS groups, further underscores the importance of vascular improvements provided by cocoa flavanols. While berry polyphenols excel in cellular protection, they may be less effective in isolation for enhancing overall endurance, as efficient oxygen delivery is a key determinant of performance during prolonged exercise. Interestingly, the placebo group slightly outperformed the BRS group, possibly due to psychological factors, dietary inconsistencies, or the relatively limited impact of berry supplementation alone on time to exhaustion [9]. Mixed-source polyphenol supplementation offers distinct advantages over single-source approaches by addressing multiple pathways of exercise-induced stress simultaneously. Cocoa flavanols primarily enhance vascular function and mitigate endothelial oxidative stress, while berry polyphenols focus on intracellular ROS scavenging, mitochondrial health, and lipid membrane stabilization [4]. The combination of these two sources creates a synergistic effect that amplifies their individual benefits, providing comprehensive protection against the oxidative, inflammatory, and metabolic challenges associated with exercise. Additionally, mixed supplementation reduces the risk of inter-individual variability in response, ensuring more consistent benefits across different populations.

Implication of the Study

The findings of this study have significant implications for athletes, coaches, and nutritionists seeking effective strategies to enhance endurance performance. The superior performance of the combined cocoa flavanols and berry supplementation (CFL+BRS) underscores the importance of leveraging complementary mechanisms, such as improved vascular function, enhanced antioxidant defense, and reduced inflammation, to optimize exercise outcomes. This highlights the potential of mixed-source polyphenol supplementation as a practical and scientifically supported intervention to combat the oxidative and inflammatory stresses of high-intensity and prolonged exercise. Additionally, the study emphasizes the limitations of single-source supplementation, such as cocoa flavanols or berries alone, and the critical role of dietary diversity in maximizing physiological benefits. These results could influence future dietary guidelines and supplementation practices, promoting the use of synergistic polyphenol combinations in athletic populations. Further, the study underscores the need for personalized nutrition strategies, considering factors like individual variability in polyphenol bioavailability and gut microbiome composition, to ensure consistent and effective outcomes across different individuals and athletic contexts.

CONCLUSIONS

This study demonstrates that the combination of cocoa flavanols (CFL) and berry polyphenols (BRS) is a superior intervention for enhancing time to exhaustion during exercise compared to single-source polyphenols or a placebo. The findings highlight the synergistic effects of mixed polyphenol supplementation, leveraging the complementary mechanisms of improved vascular function, enhanced antioxidant defense, and reduced inflammation. While CFL and BRS individually provide measurable benefits, their combined use delivers a broader spectrum of protection against the oxidative and inflammatory stresses associated with exercise, ultimately improving endurance and recovery. These results emphasize the importance of dietary diversity and mixed-source supplementation for athletes and active individuals, suggesting that this approach is more effective in promoting performance and recovery. The study also underscores the need for further research to explore the long-term effects, personalized responses, and bioavailability of mixed polyphenols in various athletic and general populations.

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