



The Consumption of Yellow Watermelon-plantain Juice before Anaerobic Exercise Improves Blood Glucose and Suppresses Oxidative Stress Formation in Rats

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Abstract

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BACKGROUND: Food supplementation before exercise could supply glucose for energy boosters and enhanced performance. Food supplements with potent antioxidant activity could also help ease oxidative stress formation during exercise.

AIM: This study aims to investigate yellow watermelon-plantain juice administration before anaerobic exercise on blood glucose level and its protective effect on markers of oxidative stress formation that is malondialdehyde (MDA) serum level.

METHODS: Thirty-five male Sprague Dawley rats were divided into five different groups in which received different treatments. Anaerobic exercise in this study was set as a swimming test for 3 min. Blood glucose levels were examined as a baseline, 13 min after juice supplementation, and after the exercise test.

RESULTS: P2 groups have the highest blood glucose level before and after exercise (111.86 mg/dl and 100.52 mg/dl, respectively). Mda levels after exercise differ significantly between groups ($p < 0.05$) with the negative control group having the highest level of Mda (7.68 nmol/ml) and P2 having the lowest level among treatment groups (1.8 nmol/ml).

CONCLUSION: It can be concluded that yellow watermelon-plantain juice supplementation before anaerobic exercise is an effective source of energy due to its rapid glucose availability in the serum. The antioxidant content in yellow watermelon-plantain juice could also suppress MDA serum level after exercise.

Introduction

Carbohydrate intake before anaerobic exercise has been the subject of research due to its potential to provide energy in skeletal muscle metabolism during exercise. Blood glucose level, derived from glycolysis and glycogenolysis after carbohydrate ingestion, is one of the main focuses to provide fuel for skeletal muscle contraction. There is some evidence that nutritional intervention before anaerobic exercise could help enhance total work capacity, by providing energy metabolism advantage during exercise [1], [2]. This was also believed that could lead to athlete performance improvement in speed and strength.

However, anaerobic exercise has an unavoidable side effect that is the production of oxidative stress due to vigorous movement, which might induce muscle damage. Various human tissues constantly produce free radicals and this production is elevated during physiological changes in high-intensity short-duration exercise [3]. Some studies reported the elevation of pro-inflammatory cytokines such as IL-6, TNF- α , and other oxidative markers such as asprosin

and irisin immediately after, during an early period of recovery, or 24 h after exercise completion [4]. This condition provokes unbalanced free radicals generated from anaerobic metabolism that overcomes natural antioxidant defense systems, such as *superoxide dismutase* and *glutathione* [5], [6].

This study is aiming to investigate the effect of acute supplementation of high-glucose food products on blood glucose levels, as well as how it may deliver a protective effect toward oxidative stress production during exercise. This, hypothetically, might consequently affect performance improvement and muscular endurance during exercise.

Yellow watermelon and ripe plantain were chosen as the main food materials used in this study. Both foods have been previously reported to help improve exercise and training performance after ingestion. Plantain and yellow watermelon were known for endurance training improvement and muscle fatigue index due to their high citrulline contents [7], [8] However, not many studies have analyzed the effect of combined yellow watermelon-plantain juice toward blood glucose level and antioxidant effect during exercise.

Many studies have reported citrulline content in watermelon to delay muscle fatigue and suppress blood lactic acid formation during exercise; however, its high water and glucose contents were also beneficial to provide energy and improve performance during anaerobic exercise. It was reported that yellow watermelon had water contents for 91.82% and 67.75% b/b in its mesocarp [9]. It was reported that yellow watermelon had water contents of 91.82% b/b in its flesh and 67.75% b/b in its mesokarp [9], whereas the glucose content was approximately 7-10% b/b [10]. Meanwhile, plantain is often linked as a quick source of energy during exercise since it helps boost energy up rapidly due to its high carbohydrate and starch contents as well as Vitamin B complex and potassium [11], [12]. These reports might suggest a beneficial effect of the yellow watermelon-plantain combination on improving performance by providing glucose as a source of energy during anaerobic exercise.

On the other hand, not many studies have reported the antioxidant content of yellow watermelon and plantain. Since oxidative stress could naturally occur after exercise, it is important to acknowledge the promising effect of yellow watermelon juice supplementation before anaerobic exercise as an antioxidant-rich supplement to suppress oxidative stress marker formation.

This study also intended to investigate the comparison of glucose utilization efficacy between energy derived from natural glucose content in food with energy sourced from added sugar during exercise. Thus, one of our treatment groups received added sugar as a substitute equivalent with glucose content in a single dose of yellow watermelon-plantain juice. In addition, we tried to examine whether the double dose of yellow watermelon-plantain juice will consequently double up its glucose availability and improve anaerobic performance, as well as improve the protective effect toward oxidative stress formation during exercise.

Methods

Design, location, and time

This study was a true experimental with pre- and post-randomized controlled group design, which was conducted in Food and Nutrition Research Centre Inter-Laboratory Universitas Gadjah Mada (UGM) in May 2019. Ethical research for this study was approved by the Research Ethics Committee with reference number 2190/KEPK/V/2019.

Materials

The yellow watermelon as one of the main ingredients used in this study was classified as

black-orange type (*Citrullus lanatus* "Black Orange"), which was obtained from yellow watermelon plantation, Nusawungu, Cilacap, Central Java, Indonesia. Ripe yellow watermelon approximately aged 70 days after planting time was chosen, weighing between 4.0 and 5.0 kg. Meanwhile, the plantain banana used in this study was classified as *Musa paradisiaca* Linn, which was obtained from plantain plantation, Kalimanah, Purbalingga, Central Java, Indonesia. Ripe plantain was characterized as yellow-colored, weighing 150–200 g of each fruit, and aged approximately 7 days after plantation time. Total glucose content from yellow watermelon and plantain harvested in this study was measured in Food and Nutrition Research Centre Inter-Laboratory UGM with analysis certificate number PS/157/V/2019. The total glucose contents for yellow watermelon and plantain were 5.19% and 15.40%, respectively.

Yellow watermelon-plantain juice

The juice products were made by extracting the yellow watermelon and plantain, creating a solid form of two products combined in a proportion of 1:1. The yellow watermelon and plantain extract were weighed for 1.8 g and 3.6 g for single and double dose, respectively. The solid form was then diluted in 200 ml of water each to create the final juice products. The double dose of supplementation had 2-timed higher glucose content than the single dose. However, as the third treatment, a single dose of extract was added with 0.27 g of granulated sugar equivalent to glucose content for a single dose of juice supplementation. We tried to compare whether there might be a different effect for the blood glucose level after supplementation between juice (natural glucose) and granulated sugar (added glucose).

Animal models

A total of 35 male Sprague Dawley rats (*Rattus Copernicus*) aged 8 weeks weighing approximately 230 g were bought from Food and Nutrition Research Centre Inter-Laboratory, Universitas Gadjah Mada. The animal laboratory was caged and maintained in the room with 12 h light/dark cycle and was fed with standard Comfeed AD for 3 days during the acclimatization period. The 4th day was the commencement of the intervention period.

Experimental design

We divided that the groups into five different categories with seven rats were randomly assigned into each group that received different treatments as shown in Table 1A.

Table 1A: Designated group and treatment

Groups	Category	Treatment	Exercise
K ₊	Positive control	No juice supplementation	No
K ₋	Negative control	No juice supplementation	Yes
P1	Treatment 1	Single-dose supplementation (1.8 g extract diluted in 200 ml of water)	Yes
P2	Treatment 2	Double-dose supplementation (3.6 g extract diluted in 200 ml of water)	Yes
P3	Treatment 3	Single-dose supplementation (1.8 g extract diluted in 200 ml of water) added with 0.27 g of granulated sugar	Yes

Exercise treatment

Thirty min after juice administration, the anaerobic exercise was conducted set as a swimming test, where rats were drowned in a water pool and were left to swim for 3 min. Afterward, the rats were pulled up from the pool and a post-exercise blood sample was taken.

Serum analysis

Blood sample collection was divided into 3 different times, which are T₀ (baseline, before juice administration), T₁ (30 min after juice supplementation, just before the exercise test begins), and T₂ (immediately after 3 min of exercise) as shown in Table 2. Blood serum was collected through rats' orbital sinus to measure blood glucose and malondialdehyde (MDA) levels. Blood glucose analysis was required to analyze fluctuated change resulting from different doses of yellow watermelon-plantain juice administration specifically in treatment groups, as well as blood glucose level after exercise compared between treatment groups and control groups. In addition, to analyze oxidative stress produced because of exercising, MDA levels were also measured immediately after exercising. Control group MDA level results were set as data comparison with the treatment group results.

Data analysis

Data obtained were analyzed using SPSS 20 for Windows (SPSS Inc., Chicago, IL). The data were presented as mean ± standard deviation. One-way analysis of variance followed with *post hoc* LSD test was applied to identify statistical differences between groups. Paired t-test was also applied to analyze blood glucose levels before and after exercise in treatment groups with a confidence level of 95%. Statistically, a significant result was considered at $p < 0.05$.

Table 2: Blood glucose level changes between baseline and after supplementation

Groups	Blood glucose T ₀ Mean ± SD (mg/dl)	Blood glucose T ₁ (mg/dl)	p-value ¹	ΔBlood glucose Mean ± SD (mg/dl)	p-value ²	
P1	67.41 ± 2.14	103.15 ± 2.89	0.000*	35.74 ± 4.05	0.002 ^{3a}	0.084 ^c
P2	67.10 ± 1.58	111.86 ± 3.13	0.000*	44.76 ± 4.47	0.004 ^{3a}	
P3	67.82 ± 2.25	105.88 ± 2.53	0.000*	38.06 ± 2.22		

p¹: statistically significant a: P1 versus P2, 1: Paired t-test b: P2 versus P3, 2: Independent t-test c: P1 versus P3, SD: Standard deviation.

Table 3: Blood level measurement post-supplementation and post-exercise

Groups	Blood Glucose T ₁ Mean ± SD (mg/dl)	p-value ²		Blood Glucose T ₂ Mean ± SD (mg/dl)	p-value ²	
P1	103.15 ± 2.89	0.000 ^{3a}	0.084 ^c	97.16 ± 2.33	0.13 ^{3a}	0.123 ^c
P2	111.86 ± 3.13	0.002 ^{3a}		100.52 ± 1.92	0.000 ^{3a}	
P3	105.88 ± 2.53			95.36 ± 1.69		

p¹: statistically significant a: P1 versus P2, 2: Independent t-test b: P2 versus P3, c: P1 versus P3, SD: Standard deviation.

Results

Blood glucose level

We presented the blood glucose level data in 3 different time measurements that were T₀ as a baseline, T₁ as 30 min after juice supplementation, and T₂ as immediately after exercise. Shapiro–Wilk normality test shows that the baseline data were distributed normally with $p > 0.05$, and Levene's test shows the distribution homogeneity with no outliers found in all groups. This indicates that our groups started this experiment in a similar condition. The independent t-test result also reported no significant differences for the blood glucose level in T₀ measurement in all groups with $p > 0.05$ as shown in Table 1B.

Table 1B: Blood glucose baseline level in all groups

Groups	Blood Glucose T ₀ Mean ± SD (mg/dl)
K ₊	65.60 ± 2.31
K ₋	66.89 ± 2.10
P1	67.41 ± 2.14
P2	67.10 ± 1.58
P3	67.82 ± 2.25

SD: Standard deviation.

Table 2 illustrates that the blood glucose level changes from the baseline to 30 min after juice supplementation. There were significant changes in blood glucose levels before and after consumption of juice in all groups ($p < 0.05$), with the highest change, occurred in the P2 group. There were also significantly different blood glucose levels before and after juice supplementation between the P1 and P2 groups and P2 and P3 groups. Meanwhile, there was no significant difference between the P1 and P3 groups.

Independent t-test results showed significant differences in blood glucose level in T₁ between P1 and P2 groups and between P2 and P3 groups ($p < 0.05$). However, there was no significant difference between the P1 and P3 groups ($p > 0.05$) as shown in Table 3. A similar result happened during T₂ blood glucose measurement with significant differences only occurring between P1 and P2, as well as P2 and P3 groups ($p < 0.05$).

Table 4 presents blood glucose level changes before and after exercise. There were significant changes in before and after exercise measurement in all groups with $p = < 0.05$. The highest blood glucose

Table 4: Blood glucose level changes before and after exercise

Groups	Blood glucose T ₁ Mean ± SD (mg/dl)	Blood glucose T ₂ Mean ± SD (mg/dl)	p-value ^a	ΔBlood Glucose T ₁ T ₂ Mean ± SD (mg/dl)	p-value ^b
K ₋	-	66.84 ± 1.67			
P1	103.15 ± 2.89	97.16 ± 2.33	0.000*	-5.98 ± 1.44	0.000** 0.006**
P2	111.86 ± 3.13	100.52 ± 1.92	0.000*	-11.34 ± 2.37	0.604 ^b
P3	105.88 ± 2.53	95.36 ± 1.69	0.000*	-10.52 ± 3.33	

p*: statistically significant a: P1 versus P2, 1: Paired t-test b: P2 versus P3, 2: Independent t-test c: P1 versus P3, SD: Standard deviation.

level change occurs in the P2 group with 11.34 mg/dl decreased. There were significant differences in the changes between P1 and P2 and P1 and P3 with $p < 0.05$. The blood glucose level in K₋ the group also differs significantly with all groups ($p < 0.05$).

MDA level

Table 5 reports the result of oxidative stress formation marker (MDA) after exercise. There were significant differences in MDA level after exercise between all groups with $p < 0.05$. The highest MDA level was found in K₋ group, which experienced exercise without given supplementation. The lowest MDA level after exercise occurred in the P2 group, which was given a double dose of juice supplementation before exercise.

Table 5: Malondialdehyde level before and after exercise

Groups	MDA Mean ± SD (nmol/ml)
Control	
K ₋	1.11 ± 0.21
K ₊	7.68 ± 0.33
Treatment	
P1	3.73 ± 0.26
P2	1.8 ± 0.41
P3	3.08 ± 0.18

SD: Standard deviation.

Discussion

It is expected that this study's results could help provide insights on promising food supplementation before exercise as a source of glucose to supply caloric and strength in anaerobic exercise, as well as help suppress oxidative stress formation after exercise. Baseline levels for blood glucose levels did not differ significantly between all groups, showing all rats in this study started the test in similar conditions and exposure. The homogeneous blood glucose level baseline showed the normal blood glucose level when the rats were not exercising or receiving any foods beforehand.

All treatment groups received yellow watermelon-plantain juice combinations in different designated doses. All rats experienced a significant increase in blood glucose 30 min afterward, as predicted. All supplementation in the treatment group contains a significant amount of carbohydrates and glucose itself, which is supposed to elevate the blood glucose level after consumption. The highest increase of blood glucose level happened in the P2 group that received 3.6 g extract of yellow watermelon-plantain in the making of juice supplementation. The highest

concentration of natural glucose provided by the yellow watermelon-plantain juice in the P2 group influences a rise in blood glucose level after consumption more than the amount of added sugar in the P3 group. Thus, we suggested athletes consume juice supplementation in the highest concentrate recommendation than adding sugar in their juice.

Afterward, the rats were treated for a swimming test. Three min later, the exercise was ended and another blood glucose level was tested. There was a significant decrease in blood glucose levels before and after the test, indicating that the blood glucose was used to provide a sudden burst of energy requirement during swimming. The highest decrease was found in the P2 group with -11.34 mg/dl. However, even this group experienced the biggest drop in blood glucose level, its final blood glucose was still the highest compared to the P1 and P3 groups.

Blood glucose transport to the muscular cell was controlled by molecular signaling pathways stimulated by exercise. Insulin signaling which is involved in GLUT4 translocation was also activated by acute muscle contraction [13]. This might explain the modulation of blood glucose uptake on muscle contraction during the swimming test as an acute exercise. Glucose uptake by muscular cells might be more effective if the glucose available is derived naturally from the juice, compared to glucose provided by added glucose. Effective glucose uptake is important to provide speed and strength in a short period and could still supply energy even more if the exercise continues.

The lowest glucose uptake happened in the P1 group that supposedly explained its less effective effect to provide energy in acute exercise. The glucose availability in the blood might affect this condition. Since the elevation of blood glucose level in the P1 group is less than the other group, glucose uptake by the muscular cells might be less since the body's blood glucose homeostasis requires a minimum level to maintain its stability. This could indicate that a lower dose of juice supplementation leads rats to get tired more easily than a higher dose due to its lower energy supply.

The present of the K₋ group was to analyze how the blood glucose level after exercise would be without prior juice supplementation. The results had no difference with baseline blood glucose level, suggesting that the rats used up all its available glucose obtained from glycogenolysis. During the fasting state, glycogenolysis plays an important role which breaking down glycogen into glucose-1 phosphate. It happened in the liver to maintain blood glucose level homeostasis as

well as in the muscle to maintain energy for contraction [14], [15], [16]. However, this condition relies on the amount of glycogen storage. If the rats have used up all their glycogen, there is a high possibility that the rats have limited performance on exercise due to unavailable glucose as the main source of energy. Thus, acute supplementation of yellow watermelon-plantain juice is important to provide additional glucose before exercise.

The production of oxidative stress is an unavoidable side effect during anaerobic exercise due to its sudden burst of high energy requirement and muscular contraction. Oxidative stress during exercise was initiated through pro-oxidant cellular production because of high-intensity exercise (Moflehi *et al.*, 2013; Park and Kwak, 2016; Kawamura and Muraoka, 2018; and Simioni *et al.*, 2018). This condition leads to cellular damage in which might trigger modification and impair various macromolecules metabolisms such as protein, and lipid. Some studies have suggested that exercise-induced oxidative stress can be attenuated by intense short to moderate intensive training to induce muscle adaptation [17], [18], [19]. However, some also suggested that consumption of exogenous antioxidants through diet before exercise could also help alleviate this condition [20], [21].

In this study, the K₊ group represents a normal condition in which rats did not participate in anaerobic exercise. K₋ group represents a condition in which rats exercise without being given antioxidant-rich juice. MDA serum level after exercise in K₋ group demonstrates a significant increase compared to non-exercise by 6.57 nmol/ml. Three min of exercise in rats is sufficient to produce oxidative stress probably due to intense physical stressors. Our study finding is in conjunction with a previous study that reported increased MDA levels in the male subject even in low-intensity modes of exercise [15]. On the contrary, another study in which compare oxidative stress status in three distinct groups of exercise showed a significant increase of MDA level only in the untrained group following graded exercise test but not in the anaerobic athlete group [3].

All treatment groups in the present study had significantly lower MDA serum levels compared with K₋ group, with the lowest being in the P2 group in which receive the highest dose of yellow watermelon-plantain juice. MDA serum level in the P2 group, however, is still significantly different than normal MDA serum level as shown in the K₊ group. This indicates that even though supplementation of exogenous antioxidants might have taken place before exercise, oxidative stress formation still occurred during intense muscle contraction and could not be fully attenuated. However, yellow watermelon-plantain juice is highly effective to suppress the MDA formation if consumed inappropriate amount.

The suppression of MDA formation in this study might result from potent antioxidants contained in yellow watermelon, such as carotenoids, lycopene, phenol, flavonoid, and ascorbic acid [22], [23]. Such antioxidant

compound was quite similar to flavonoid compounds in pomegranate, which were also found to be a potent MDA level suppression formed during exercise [24]. Meanwhile, plantain banana was also contained high levels of antioxidants such as galliccatechin [25], phenolic, and flavonoid [26].

Conclusion

We concluded that consumption of yellow watermelon-plantain juice with the manufacturing process as stated in this study is effective to supply energy during anaerobic exercise, as well as suppressing MDA serum level. Further studies might require helping explain the molecular pathway for these findings.

Ethical statement

This study has followed the institutional guidelines for the care and the use of laboratory animals.

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