

BUKTI KORESPONDENSI
ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul Artikel : Effect of isolated soy protein ingestion combined with different types of carbohydrates on muscle fatigue recovery in rat exercise model

Jurnal : Sport Scinces for Health

Penulis : Mardiana, Dyah Mahendrasari, Latifah Rachmawati, Safrina
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No	Perihal	Tanggal
1.	Bukti article submission to “Sport Sciences for Health” Journal	05 Desember 2022
2.	Bukti technical check (05-12-2022)	05 Desember 2022
3.	Bukti admendment submission	11 Desember 2022
4.	Bukti peer review: first revision	13 Desember 2022
5.	Bukti revised version submission	04 Maret 2023
6.	Bukti peer review: proofread document attachment	11 Maret 2023
7.	Bukti proofread document submission	13 Maret 2023
8.	Bukti article accepted	02 Juni 2023
9.	Bukti article publish	08 Juni 2023

1. Bukti article submission to “Sport Sciences for Health” Journal (05-12-2022)

Submission received

Submission received

05 Dec 2022

2. Bukti technical check (05-12-2022)

Sport Sciences for Health : Amendment required Kotak Masuk x



Hema Shree Thirunavukarasu <hemashree.thirunavukarasu@springernature.com>
kepada saya ▾

Sen, 5 Des 2022, 21.12 ★ 😊 ↶ ⋮

[Terjemahkan ke Indonesia](#) ×

Dear Dr. Oksidriyani,

Re: Effect of isolated soy protein ingestion combined with different types of carbohydrates on muscle fatigue recovery in rat exercise model

Our Initial Quality Check of your submission has now taken place. As a result, we need you to address the following points before your manuscript can progress any further:

- Funding declaration is missing in the manuscript. Please check.
- We notice that conflict of interest is not provided in the manuscript. Could you please check?
- Author Contribution is missing for the given authors. Please check.

Your paper has been placed back in the menu of the submitting author. To access it, please use the following link, making sure you log in with the same email address you registered with:

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Please make the requested amendments carefully, before selecting the "Submit manuscript" button on the "Review" page. Do not change anything else in your manuscript.

Meanwhile, if you have any questions, please feel free to contact me.

Best regards,

Hema

3. Bukti Admendment submission (11-12-2022)

Technical check

Submission passed technical check 13 Dec 2022

Amendment received 11 Dec 2022

Submission is under technical check 05 Dec 2022

4. Bukti Peer review: first revision (13-12-2022)

Reviewer's Comments to The Author(s)

Dear author(s), I have read your study with great interest. First of all, I want to thank you for your effort to investigate the beneficial methods for muscle recovery after training. Although I like your approach to this subject, I believe that this study can contribute to the literature if you complete the deficiencies in the methodology of your research. I will list some points below that will strengthen your study in my opinion.

General Requirements;

- First of all, there are typos throughout the article, you should correct them.
- Once you have shown an abbreviation term in the text, it would be more appropriate to use only its abbreviated form (for example; "lactate dehydrogenase(LDH)" use only LDH in whole text after first showing this).

Methodological Requirements (Major Revision);

- You have to specify the gender of the rats (male or female), physiological responses to external stimuli differ in terms of gender. For this reason, be sure to base your hypothesis on this basic information by specifying the gender of the sample group in your study. In addition, since 9-week-old rats are not adults yet, they cannot be physiologically evaluated as experimental reflections of adult athletes.
- There is a lack of information on how long this experimental study took to complete in total. You have to specify the total time period.
- I recommend you to explain the adaptation, feeding and experimental environment more detailed afterwards.
- Give more details about the experimental groups, it is not clear. Especially why control group has maltodextrin supplementation? This group should not have any supplementation in my opinion.
- You have to explain how you took blood samples to measure baseline LDH and BG experiment. You should specify the points like; blood samples collected from tail vein or jugular vein or cardiac punch or etc...?
- You have to specify how you decided to use Kruskal-Wallis, Friedman and Wilcoxon? Did you check the normality of the variables? You better explain this more detailed.

Discussion and Conclusion;

The discussion section is weak in comparison with the literature and should be expanded a little more. In addition, making suggestions by expanding the conclusion part takes the study to a more serious level.

I wish you good luck!

5. Bukti Revised version submission (04-03-2023)

Peer review	
Submission accepted	02 Jun 2023
Submission under peer review	11 Mar 2023
Submission passed technical check	11 Mar 2023
Revision received	04 Mar 2023
Submission under peer review	13 Dec 2022

Effect of isolated soy protein ingestion combined with different types of carbohydrates on muscle fatigue recovery in rat exercise model

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Abstract

Background: The consumption of isolated soy protein (ISP) is well known for its beneficial effect on muscle recovery. However, The effect of its combination with maltodextrin, honey, or dates has never been investigated. This study sought to determine the best combination of ISP and different types of carbohydrates for muscle fatigue recovery in the exercise-induced fatigue rat model.

Methods: After 3 days of adaptation, twenty-four 9-week-old Sprague Dawley rats were randomly assigned to 4 groups: the maltodextrin supplementation (control) group, the maltodextrin and ISP (P1) group, the honey and ISP (P2) group, and the dates and ISP (P3) group. Each group included six rats for the one-week experiment. Before the experimental period, baseline values of body weight and lactate dehydrogenase (LDH) were recorded for each group. Throughout the study, all rats performed the swimming test until exhaustion every two days. Following supplementation, LDH levels were measured three times: immediately after exercise, three hours later, and seven days later.

Results: The results showed that the mean LDH of the P2 group was significantly decreased after 3 hours (63%) and 7 days (66%) of supplementation (p 0.05) compared with the other groups.

Conclusion: The consumption of carbohydrates and ISP will accelerate muscle recovery in rats following exercise. In this study, honey was more effective than maltodextrin or dates at reducing LDH levels.

Keywords: isolated soy protein, maltodextrin, honey, dates, muscle fatigue, exercise

INTRODUCTION

Training and recovery strategies influence athlete performance. The risk of post-exercise muscle fatigue, which affects internal physiological conditions, can exist for competition activities and routine training with little time for recovery.^{1,2} Oxidative stress, glycogen, lactate, creatine kinase (CK), and LDH levels were increased.^{3,4}

Resting, massage, and proper nutrition can help reduce muscle fatigue.⁵ After exercise, nutrition can help muscles feel less achy and restore glycogen as a source of energy. These dietary requirements can be packaged in useful ways, one of which is as a sports gel drink. Carbohydrates and proteins are the nutrients that are required in this condition. Glycogen is resynthesised with the help of carbohydrates. Because it provides branched-chain amino acids, ISP aids in the regeneration of skeletal muscle cells and the restoration of glycogen.⁶

ISP and carbohydrates may reduce muscle fatigue following a high-intensity exercise by maintaining energy levels.⁷ Dates, honey, and soy protein are examples of natural sources that athletes may use as sports gel drink

supplements.⁸⁻¹² To assure the best energy restoration and effects on muscle cells, it is necessary to analyze a formula for a sports gel drink that combines soy protein with dates or honey. The purpose of this study is to evaluate the efficacy of a sports drink containing ISP and various types of carbohydrates in assisting Sprague-Dawley rats to recover from post-exercise muscle fatigue.

MATERIALS AND METHODS

MATERIALS

Ingredients

The ISP used in this study was purchased from Nat Lab Indonesia. This product contains 95% ISP with no added sweeteners or flavors. The nutrients and amino acids present in the ISP were analyzed by NatLab Indonesia.

Animals and Grouping

A 9-week-old male Sprague Dawley rat weighing between 185 and 200 g from the Food and Nutrition Laboratory at Gadjah Mada University in Yogyakarta, Indonesia, was used in this experiment. Rats were fed a standard mouse chow diet with a basal diet and water ad libitum. The 24 rats were acclimatized for three days to allow them

to adapt to the feeding environment and swimming exercise before being randomly divided into four equal-sized groups: the maltodextrin supplementation (control) group, the maltodextrin and ISP (P1) group, the honey and ISP (P2) group, and the dates and ISP (P3) group. The administration of maltodextrin to the control group was intended to reflect the actual post-exercise state of athletes, with maltodextrin consumption destined to provide athletes with rapid energy after a reduction in blood sugar levels.

We conducted the standard daily operation for seven days, which included a standard diet quota, free drinking, swimming treatment every two days, and carbohydrate-protein supplementation after swimming treatment.

METHODS

Exhaustive Swimming Test

Each rat was individually placed in a 50 cm x 40 cm x 35 cm swimming pool that was filled with water to a depth of 35 cm and maintained under standard laboratory conditions (25 °C controlled temperature, 12-hour light/dark cycle, and 70% relative humidity). Loss of coordination and the inability to return to

the surface within 10 seconds were indicators of exhaustion. The rats were then removed from the pool, dried with paper towels, and placed back in their cages. After each session, the water in the pool was replaced.

Supplementation

The control group was given 0,9 g of maltodextrin per 200 g of body weight. The P1 group was administered 0,9 g of maltodextrin and 1,29 g of ISP per 200 g of body weight. The P2 group was administered 1,04 g of honey and 1,29 g of ISP per 200 g of body weight. The P3 group was administered 1,15 g of dates and 1,29 g of ISP per 200 g of body weight. Supplements were given to the rats following their exhaustive swimming treatment.

LDH Measurement

Following three days of acclimatization, we determined the rats' baseline characteristics (day 0), including body weight and LDH that were obtained from eye blood samples (orbital sinus). After obtaining baseline characteristics, LDH levels were measured three times in each rat: immediately after exercise (day 1), three

Discussion

Immediately following the swimming exercise and carbohydrate-protein supplement interventions, LDH levels increased in every treatment group. This may have occurred as a consequence of intensive swimming training that led to lactic acid accumulation, as the supplement has yet to influence lactate conversion into glucose.¹⁵

LDH levels began decreasing three hours after ingestion. This result was consistent with the findings of [Tsintzas et al.](#), who demonstrated that consuming 175 g carbohydrate during the 4 hours of recovery from running exercise resulted in greater muscle glycogen resynthesis that [inturn](#) decrease the LDH level than consuming 50 g carbohydrate.^{15, 16}

On the seventh day, LDH levels in all groups decreased significantly, including the control group, which received only maltodextrin. This might be caused by a combination of recovery time and nutrition.¹⁷ Athletes must strike a balance between stress (such as training and competition loads) and recovery in order to continue performing at a high level (like sleep and cold water immersion). The athlete's nutrition plan,

on the other hand, is one of the most important ways to speed up muscle recovery and improve performance.¹⁸ Carbohydrate consumption during the initial phase of post-exercise recovery is one of the most important factors in maximizing recovery time. To aid in the synthesis of muscle glycogen, foods with a moderate to high glycemic index provide athletes with readily available carbohydrates.^{19,20} ISP's content of branched-chain amino acids (BCAA) can reduce muscle deterioration and accelerate muscle repair.⁹

The P2 group, which received honey and ISP, experienced the greatest change in LDH levels. Numerous studies have examined the inclusion of honey in sports nutrition formulations.¹⁹ Honey assists athletes in maintaining a healthy nutritional balance and maximizing their performance. In terms of natural sweetness, honey is superior to other sweeteners that are typically added to foods and beverages for athletes. In addition, honey is rich in vitamins, minerals, and bioactive compounds, which can increase the product's value.^{20,22}

Conclusion

Carbohydrate consumption and ISP will accelerate muscle recovery after exercise in rats. In this study, honey was more effective at reducing LDH levels than maltodextrin or dates.

Conflict of Interest

We declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Dual Publication

The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration (from you or one of your Contributing Authors) by another publisher.

Author Contribution

M.M. and D.M. conceived of the presented idea. T.A., V.J., and D.V. developed the theory and performed the computations. M.M. and S.O. verified the analytical method. L.R. and S.O. carried out the experiment. S.O. write the manuscript with support from M.M. All

author discussed the result and contributed to the final manuscript.

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6. Bukti Peer review (11-03-2023)

Peer review	
Submission accepted	02 Jun 2023
Submission under peer review	11 Mar 2023
Submission passed technical check	11 Mar 2023
Revision received	04 Mar 2023
Submission under peer review	13 Dec 2022

7. Bukti Article accepted (02-06-2023)

Peer review	
Submission accepted	02 Jun 2023
Submission under peer review	11 Mar 2023
Submission passed technical check	11 Mar 2023
Revision received	04 Mar 2023
Submission under peer review	13 Dec 2022

8. Bukti Article publish (08-06-2023)

Publishing and rights

Publishing and rights complete 08 Jun 2023

Submission is in publishing and rights 08 Jun 2023

Sport Sciences for Health
<https://doi.org/10.1007/s11332-023-01080-w>

RESEARCH



Effect of isolated soy protein ingestion combined with different types of carbohydrates on muscle fatigue recovery in rat exercise model

Mardiana¹ · Dyah Mahendrasari¹ · Latifah Rachmawati¹ · Safrina Oksidriyani¹ · Safira Chairani Dimarti¹ · Tsaniatin N. Al Amien¹ · Vitta M. B. Jayaputra¹ · Dyan Violeta¹

Received: 5 December 2022 / Accepted: 2 June 2023
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Abstract

Background The consumption of isolated soy protein (ISP) is well known for its beneficial effect on muscle recovery. However, the effect of its combination with maltodextrin, honey, or dates has never been investigated. This study sought to determine the best combination of ISP and different types of carbohydrates for muscle fatigue recovery in the exercise-induced fatigue rat model.

Methods After 3 days of adaptation, 24 9-week-old Sprague Dawley rats were randomly assigned to four groups: the maltodextrin supplementation (control) group, the maltodextrin and ISP (P1) group, the honey and ISP (P2) group, and the dates and ISP (P3) group. Each group included six rats for the 1-week experiment. Before the experimental period, baseline values of body weight and lactate dehydrogenase (LDH) were recorded for each group. Throughout the study, all rats performed the swimming test until exhaustion every 2 days. Following supplementation, LDH levels were measured three times: immediately after exercise, 3 h later, and 7 days later.

Results The results showed that the mean LDH of the P2 group was significantly decreased after 3 h (63%) and 7 days (66%) of supplementation ($p = 0.05$) compared with the other groups.

Conclusion Ingestion of ISP combined with honey after exercise improves muscle recovery in rats.

Keywords Isolated soy protein · Maltodextrin · Honey · Dates · Muscle fatigue · Exercise

Introduction

Training and recovery strategies influence athlete performance. The risk of post-exercise muscle fatigue, which affects internal physiological conditions, can exist for competition activities and routine training with little time for recovery [1, 2]. Oxidative stress, glycogen, lactate, creatine kinase (CK), and LDH levels were increased [3, 4].

Resting, massage, and proper nutrition can help reduce muscle fatigue [5]. After exercise, nutrition can help muscles feel less achy and restore glycogen as a source of energy. These dietary requirements can be packaged in useful ways, one of which is as a sports gel drink. Carbohydrates and proteins are the nutrients that are required in this condition. Glycogen is resynthesized with the help of carbohydrates.

Because it provides branched-chain amino acids, ISP aids in the regeneration of skeletal muscle cells and the restoration of glycogen [6].

ISP and carbohydrates may reduce muscle fatigue following a high-intensity exercise by maintaining energy levels [7]. Dates, honey, and soy protein are examples of natural sources that athletes may use as sports gel drink supplements [8–12]. To assure the best energy restoration and effects on muscle cells, it is necessary to analyze a formula for a sports gel drink that combines soy protein with dates or honey. The purpose of this study is to evaluate the efficacy of a sports drink containing ISP and various types of carbohydrates in assisting Sprague–Dawley rats to recover from post-exercise muscle fatigue.

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¹ Department of Nutrition, Faculty of Medicine, Universitas Negeri Semarang, Semarang, Indonesia

Materials and methods

Materials

Ingredients

The ISP used in this study was purchased from Nat Lab Indonesia. This product contains 95% ISP with no added sweeteners or flavors. The nutrients and amino acids present in the ISP were analyzed by NatLab Indonesia.

Animals and grouping

A 9-week-old male Sprague–Dawley rat weighing between 185 and 200 g from the Food and Nutrition Laboratory at Gadjah Mada University in Yogyakarta, Indonesia, was used in this experiment. Rats were fed a standard mouse chow diet with a basal diet and water ad libitum. The 24 rats were acclimatized for 3 days to allow them to adapt to the feeding environment and swimming exercise before being randomly divided into four equal-sized groups: the maltodextrin supplementation (control) group, the maltodextrin and ISP (P1) group, the honey and ISP (P2) group, and the dates and ISP (P3) group. The administration of maltodextrin to the control group was intended to reflect the actual post-exercise state of athletes, with maltodextrin consumption destined to provide athletes with rapid energy after a reduction in blood sugar levels.

We conducted the standard daily operation for 7 days, which included a standard diet quota, free drinking, swimming treatment every 2 days, and carbohydrate–protein supplementation after swimming treatment.

Methods

Exhaustive swimming test

Each rat was individually placed in a 50 cm × 40 cm × 35 cm swimming pool that was filled with water to a depth of 35 cm and maintained under standard laboratory conditions (25 °C controlled temperature, 12-h light/dark cycle, and 70% relative humidity). Loss of coordination and the inability to return to the surface within 10 s were indicators of exhaustion. The rats were then removed from the pool, dried with paper towels, and placed back in their cages. After each session, the water in the pool was replaced.

Supplementation

The control group was given 0.9 g of maltodextrin per 200 g of body weight. The P1 group was administered 0.9 g of maltodextrin and 1.29 g of ISP per 200 g of body weight. The P2 group was administered 1.04 g of honey and 1.29 g of ISP per 200 g of body weight. The P3 group was administered 1.15 g of dates and 1.29 g of ISP per 200 g of body weight. Supplements were given to the rats following their exhaustive swimming treatment.

LDH measurement

Following 3 days of acclimatization, we determined the rats' baseline characteristics (day 0), including body weight, and LDH that were obtained from eye blood samples (orbital sinus). After obtaining baseline characteristics, LDH levels were measured three times in each rat: immediately after exercise (day 1), 3 h later (day 1), and 7 days later (day 7).

Statistic analysis

The data are presented as means ± standard errors (SEs). After conducting normality check, statistical test was performed using Statistical Product and Service Solutions (SPSS) to determine the difference. The ANOVA test is performed if the assumption of normality is met. If this condition is not met, the Kruskal–Wallis test is used. *p* values less than 0.05 were considered significant.

Results

In both humans and animals, acute exercise can cause tissue or cellular membrane damage and result in the release of cellular contents into the plasma [13, 14]. Some indicators, such as LDH, can be used to identify these phenomena. We collected blood samples three times during the intervention week to track the dynamic changes in LDH activity following carbohydrate–protein supplementation via tube feeding.

LDH levels improved in all groups when measured immediately after exercise and supplementation. LDH levels decreased significantly 3 h after the intervention in the P1, P2, and P3 groups ($p < 0.05$), with the P2 group experiencing the greatest average decrease, which was approximately 63% lower than the previous measurement results ($p = 0.01$). LDH activity in the P2 group also decreased significantly below baseline levels after a 7-day intervention (Table 1).

Table 1 Effect of intervention on LDH levels (U/l)

	Control	P1	P2	P3	<i>p</i> value
Pre-test	90.06 ± 3.11	90.06 ± 7.11	95.57 ± 3.11	91.44 ± 5.48	0.214 ^a
Post 0-h	277.06 ± 4.06	277.75 ± 5.64	271.57 ± 14.62	279.13 ± 4.26	0.677 ^b
Post 3-h	281.88 ± 3.37	162.25 ± 8.91	100.38 ± 8.11	118.25 ± 6.21	< 0.001 ^a
Post 7-d	235.13 ± 9.25	149.88 ± 9.65	91.44 ± 6.61	110.00 ± 5.00	< 0.001 ^a
Δ_1	4.82 ± 1.68	-115.50 ± 4.52	-171.19 ± 18.95	-160.88 ± 3.69	< 0.001 ^b
Δ_2	-46.75 ± 7.22	-12.37 ± 3.69	-8.94 ± 4.05	-8.25 ± 2.61	0.002 ^b

Each value represent means ± SE. *n* = 6. $\Delta_1 = \text{LDH (3-h)} - \text{LDH (0-h)}$, $\Delta_2 = \text{LDH (7-d)} - \text{LDH (3-h)}$

Pre-test: before intervention; 0-h: immediately after intervention; 3-h: 3 h after intervention; 7-d: 7 days after intervention. Intervention included swimming exercise and carbohydrate-protein supplementation

^aANOVA test; ^bKruskal-Wallis test

Discussion

Immediately following the swimming exercise and carbohydrate-protein supplement interventions, LDH levels increased in every treatment group. This may have occurred as a consequence of intensive swimming training that led to lactic acid accumulation, as the supplement has yet to influence lactate conversion into glucose [15].

LDH levels began decreasing 3 h after ingestion. This result was consistent with the findings of Tsintzas et al., who demonstrated that consuming 175 g carbohydrate during the 4 h of recovery from running exercise resulted in greater muscle glycogen resynthesis that in turn decrease the LDH level than consuming 50 g carbohydrate [15, 16].

On the seventh day, LDH levels in all groups decreased significantly, including the control group, which received only maltodextrin. This might be caused by a combination of recovery time and nutrition [17]. Athletes must strike a balance between stress (such as training and competition loads) and recovery to continue performing at a high level (like sleep and cold water immersion). The athlete's nutrition plan, on the other hand, is one of the most important ways to speed up muscle recovery and improve performance [18]. Carbohydrate consumption during the initial phase of post-exercise recovery is one of the most important factors in maximizing recovery time. To aid in the synthesis of muscle glycogen, foods with a moderate to high glycemic index provide athletes with readily available carbohydrates [19, 20]. ISP's content of branched-chain amino acids (BCAA) can reduce muscle deterioration and accelerate muscle repair [9].

The P2 group, which received honey and ISP, experienced the greatest change in LDH levels. Numerous studies have examined the inclusion of honey in sports nutrition formulations [21]. Honey assists athletes in maintaining a healthy nutritional balance and maximizing their performance. In terms of natural sweetness, honey is superior to other sweeteners that are typically added to foods and beverages for athletes. In addition, honey is rich in vitamins,

minerals, and bioactive compounds, which can increase the product's value [20, 22].

Conclusion

Carbohydrate consumption and ISP will accelerate muscle recovery after exercise in rats. In this study, honey was more effective at reducing LDH levels than maltodextrin or dates.

Author contributions MM and DM conceived of the presented idea. TA, VJ, and DV developed the theory and performed the computations. MM and SO verified the analytical method. LR and SCD carried out the experiment. SO wrote the manuscript with support from MM. All authors discussed the result and contributed to the final manuscript.

Data availability statement The data that support the findings of this study are available from the corresponding author, SO, upon reasonable request.

Declarations

Conflict of interest We declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Ethical approval This study was approved by the Ministry of Health of Republic Indonesia (Ethical Clearance (EC) No. 239/KEPK/EC/2022).

Statement of human and animal rights All of the experimental procedure involving animals were conducted in accordance with the experimental animal care guidelines of the Ministry of Health of Republic Indonesia.

Informed consent There are no human subjects in this experimental study and informed consent is no needed.

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