purwo sri rejeki <purwo-s-r@fk.unair.ac.id>

Chiang Mai University Journal of Natural Sciences - Manuscript ID CMUJ-2021-0218

Priraya Rithaporn <onbehalfof@manuscriptcentral.com> Tue, Sep 21, 2021 at 7:08 PM
Reply-To: prirayar@hotmail.com
To: purwo-s-r@fk.unair.ac.id, purwo_faal@yahoo.com

21-Sep-2021

Dear Dr. Rejeki:

Your manuscript entitled "Comparison Effect of Nocturnal Diurnal Moderate-intensity Swimming Exercise in Increasing Irisin Level of Female Mice (Mus musculus)" has been successfully submitted online and is presently being given full consideration for publication in the Chiang Mai University Journal of Natural Sciences.

Your manuscript ID is CMUJ-2021-0218.

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Chiang Mai University Journal of Natural Sciences - Decision on Manuscript ID CMUJ-2021-0218

Korakot Nganvongpanit <onbehalfof@manuscriptcentral.com> Tue, Nov 2, 2021 at 12:00 PM
Reply-To: korakot.n@cmu.ac.th
To: purwo-s-r@fk.unair.ac.id, purwo_faal@yahoo.com

02-Nov-2021

Dear Dr. Rejeki:

Manuscript ID CMUJ-2021-0218 entitled "Comparison Effect of Nocturnal Diurnal Moderate-intensity Swimming Exercise in Increasing Irisin Level of Female Mice (Mus musculus)" which you submitted to the Chiang Mai University Journal of Natural Sciences, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

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Once again, thank you for submitting your manuscript to the Chiang Mai University Journal of Natural Sciences and I look forward to receiving your revision.

Sincerely,
Dr. Korakot Nganvongpanit
Editor-in-Chief, Chiang Mai University Journal of Natural Sciences
korakot.n@cmu.ac.th

Note by editor: Please check also recent similar publications in “Chiang Mai University Journal of Natural Sciences”, and if you find some relevant publications, highlight your novelty compared to these previous works.
Associate Editor Comments to Author:

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
Suggesting title:
Nocturnal Moderate-intensity Swimming Exercise Increase more Irisin Level of Female Mice (Mus musculus)

INTRODUCTION:
Researchers
Researchers hypothesized that moderate-intensity swimming exercise performed at night was more effective in increasing irisin levels of female mice (Mus musculus) than in the morning. --> delete this sentence.

RESULT
Make figures less simple. please choose, positive or negative error bar caps.
Below figure, explain G1=...; G2=.... etc

DISCUSSION
please state briefly about irisin circardian rhytm.

English needs improvement.

Reviewer: 2

Comments to the Author
The author must carefully explain the methods, data/results, and the discussion section. The idea of this study is quite interesting. However, the author must pay more attention to some parts of the manuscript to avoid unclear claims and judgment based on the data. Also, the author must explain briefly the limitation of this study and the novel findings as proof of new invention within this manuscript.

Reviewer: 3

Comments to the Author
Blood samples were take 12 hr after the last exercise bout in the 4 week training program. The authors are encouraged to provide information that the irisin levels were not the result of the last exercise bout.

The irisin levels seem to be quite low compared to others studies in mice, e.g. doi: 10.1038/boneres.2016.56 and doi: 10.3390/nu9040410. The authors need to provide information that their irisin levels can be compared to other studies.

It is not clear why the exercise intensity was moderate-intensity. Please clarify.

I suggest to change in the title “Comparison Effect of Nocturnal Diurnal Moderate-intensity Swimming Exercise in Increasing” to “Effect of Nocturnal and Diurnal Moderate-intensity Swimming Exercise on”

Throughout the manuscript, I suggest to change “pre-exercise” and “post-exercise” by “pre-training” and “post-training”.

Ls13 and 90. Change “maximum time” To “maximum swimming time”.

L43. Please change “p = 0.000.” to “p < 0.001.”

L113. Data in the figures is displayed as mean and SD. Please clarify.

L142. I suggest to delete “Based on the results of the final study,”

L150. Change “Discussions” to “Discussion”.

Ls 160-161 and L165. Fatty acids are available as an energy source without the need for conversion to glucose. Please revise.

purwo sri rejeki <purwo-s-r@fk.unair.ac.id>

Chiang Mai University Journal of Natural Sciences - Decision on Manuscript ID CMUJ-2021-0218.R1

1 message

Korakot Nganvongpanit <onbehalfof@manuscriptcentral.com> Tue, Jan 4, 2022 at 11:03 AM
Reply-To: korakot.n@cmu.ac.th
To: purwo-s-r@fk.unair.ac.id, purwo_faal@yahoo.com

04-Jan-2022

Dear Dr. Rejeki:

Manuscript ID CMUJ-2021-0218.R1 entitled "Effect of Nocturnal and Diurnal Moderate-intensity Swimming Exercise on Increasing Irisin Level of Female Mice (Mus musculus)" which you submitted to the Chiang Mai University Journal of Natural Sciences, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.

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Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s). Also, please be sure to indicate by page and line number where the corresponding changes can be found in the revised version of your paper.

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

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Once again, thank you for submitting your manuscript to the Chiang Mai University Journal of Natural Sciences and I look forward to receiving your revision.

Sincerely,
Dr. Korakot Nganvongpanit
Editor-in-Chief, Chiang Mai University Journal of Natural Sciences
korakot.n@cmu.ac.th

Associate Editor Comments to Author:

Associate Editor

Comments to the Author:
(There are no comments.)

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
the author have to revise the manuscript based on the reviewer comment and supported by an adequate answer for each question/

Reviewer: 2

Comments to the Author
The irisin levels seem to be quite low compared to others studies in mice, e.g. doi: 10.1038/boneres.2016.56 and doi: 10.3390/nu9040410. The authors need to provide information that their irisin levels can be compared to other studies.

It is not clear why the exercise intensity was moderate-intensity. Please clarify.

There is still mention of fatty acids being converted to glucose during exercise. That is incorrect. Please change.
****

Editor comments
The authors have not responded to some of the essential comments from our reviewer on the first submission. This is problematic, see below which comments the authors ignored. As these are quite essential for make a decision on this manuscript.

A comment on the first submission was “Blood samples were take 12 hr after the last exercise bout in the 4 week training program. The authors are encouraged to provide information that the irisin levels were not the result of the last exercise bout.”

In the revision, this is changed to 24 hr after the last exercise bout without any explanation. The change to 24 hr is not in yellow as are other changes to the manuscript.

A comment on the first submission was “The irisin levels seem to be quite low compared to others studies in mice, e.g. doi: 10.1038/boneres.2016.56 and doi: 10.3390/nu9040410. The authors need to provide information that their irisin levels can be compared to other studies.”

In the revision, the authors have not responded to this.

A comment on the first submission was “It is not clear why the exercise intensity was moderate-intensity. Please clarify.”

In the revision, the authors have not responded to this.

A comment on the first submission was “Ls 160-161 and L165. Fatty acids are available as an energy source without the need for conversion to glucose. Please revise.”

In the revision, the authors still state that fatty acids may be converted to glucose. That is incorrect.

We need you response all comments point by point.

purwo sri rejeki <purwo-s-r@fk.unair.ac.id>

Chiang Mai University Journal of Natural Sciences - Manuscript ID CMUJ-2021-0218.R2

1 message

Priraya Rithaporn <onbehalfof@manuscriptcentral.com> Fri, Mar 4, 2022 at 11:09 PM
Reply-To: prirayar@hotmail.com
To: purwo-s-r@fk.unair.ac.id, purwo_faal@yahoo.com

05-Mar-2022

Dear Dr. Rejeki:

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Sincerely,
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Effect of Nocturnal and Diurnal Moderate-intensity Swimming Exercise on Increasing Irisin Level of Female Mice (*Mus musculus*)

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AUTHOR CONTRIBUTIONS

Conceiving and designing the experiments: MFA PSR. Performing the experiments: MFA PSR S. Analyzing the data: AP RL. Contributing reagents/materials/analysis tools: YY KWW GMS. Writing the paper: MFA PSR S AP KWW GMS YY RL.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

This study was supported by the internal research grant of the Faculty of Medicine (*Penelitian Unggulan Fakultas*), Universitas Airlangga, Surabaya, Indonesia, under Grant Number: 219/UN/3.1.1/PT/2021. We also thank to Virology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga to help in conducting this research.

Effect of Nocturnal and Diurnal Moderate-intensity Swimming Exercise on Increasing Irisin Level of Female Mice (*Mus musculus*)

ABSTRACT

This study aims to compare moderate-intensity swimming exercise in the morning and at night towards the increment of irisin levels in female mice (*Mus musculus*). This research is a real experiment with the randomized control group post-test-only design. A total of 24 female mice (*Mus musculus*), aged 3 months old, and weighing 20-40 grams (Lee's Index Value > 0.3) were randomized into three groups, namely G_1 (n = 8, control without intervention), G_2 (n = 8, moderate-intensity swimming exercise in the morning), and G_3 (n = 8, moderate-intensity swimming exercise at night). Swimming exercises were carried out 3x/w for 4 weeks with an intensity of 6% of body weight and the duration was 70% of maximum swimming time. Irisin levels were measured using the ELISA method. The data analysis techniques used were one-way ANOVA test and Tukey's HSD post-hoc test. The results that were obtained from this experimental study were mean levels of irisin in G_1 (1.86 ± 0.06 ng/mL), G_2 (2.66 ± 0.12 ng/mL), G_3 (3.43 ± 0.35 ng/mL), and ($p \leq 0.001$). The results of Tukey's HSD post-hoc test showed that there was a significant difference in the mean post-training irisin levels between G_2 and G_1 ($p \leq 0.05$), G_3 and G_1 ($p \leq 0.001$), and G_3 and G_2 ($p \leq 0.05$). Based on the results of this study, it was concluded that moderate-intensity swimming exercises in the morning and at night were able to increase irisin levels. However, moderate-intensity swimming exercise at night showed a higher effectiveness in increasing irisin levels of female mice (*Mus musculus*).

KEYWORDS: Obesity, irisin levels, morning exercise, night exercise

1. INTRODUCTIONS

Exercise is not only an active and preferred way to improve health, but also an effective way to reduce harm of metabolic diseases (Badawy et al., 2020; Smith & Adams, 2011), such as obesity and diabetes mellitus type 2 (Fatouros, 2017; Sigal et al., 2006). In addition, exercise also has a positive impact on body health because it promotes muscle contractions. Muscle contraction activates molecular pathways and myokine pathways that do not only act on muscles via autocrine or paracrine, but also mediate interactions between muscles and other organs through endocrine mechanisms (Pedersen & Febbraio, 2008). One of the myokines that play a role in preventing metabolic syndrome is irisin. Irisin is one of myokine types that is secreted by the proteolytic cleavage of membrane protein fibronectin type III domain-containing protein 5 (FNDC5) and it is regulated by peroxisome proliferation-activated receptor coactivator-1α (PGC1α) (Boström *et al*., 2012). Irisin acts on white fat cells to stimulate protein-1 uncoupling (UCP-1) and in the browning process of fat tissue (Fatouros, 2017). Irisin is released into the bloodstream to eleveate energy expenditure, therefore irisin might be used to treat obesity problems and maintain glucose homeostasis (Boström et al., 2012).

Exercise is one of the safe non-pharmacological approaches to increase irisin levels, which is a mediator of increasing energy metabolism (Fatouros, 2017). Irisin is involved in white adipose tissue browning and energy metabolism which plays an important role in increasing insulin sensitivity (Yang et al., 2016). Lower levels of irisin indicate a low metabolic rate, resulting in an increase in energy stores in body and an increased risk of obesity. Obesity and low metabolic rate conditions in individuals with low levels of physical activity (sedentary lifestyle) will have a negative impact on health (Yang et al., 2016).

Common exercise barriers include lack of time, long work hours, and demanding study during the day that causes the right time to do exercise to be at night to avoid a sedentary

lifestyle. However, according to Algul et al. (2017), the exact time to exercise for humans according to lifestyle (circadian rhythm) for health is still unknown, nor the time of exercise to burn body fat. Likewise, the exact time to exercise in mice is also not clearly known (Mendoza et al., 2021). Nocturnal mice, unlike humans, sleep during the day and behave at night (Garidou-Boof et al., 2005; Perreau-Lenz et al., 2004; Cajochen et al., 2003). The biological and psychological circadian rhythm times have a major influence on physical performance, which is most often observed in the early night by considering individual chronotypes and using timed training could be an effective method of improving physical performance at a given point in time, which will promote subcutaneous browning of white adipocytes (Teo et al., 2011; Norheim et al., 2014). In addition to the time factor, exercise intensity can also affect changes in irisin levels (Winn et al., 2017). Moderate-intensity exercise is relatively easy to do because it does not require great effort compared to high-intensity exercise (Omar *et al*., 2021).

Based on the description of the background described above, the purpose of this study was to compare moderate-intensity swimming exercises which were carried out in the morning and at night towards the increament of irisin levels in female mice (*Mus musculus*).

2. MATERIALS AND METHODS

2.1. Experimental Design

This research is a real experiment with the randomized control group post-test-only design. A total of 24 female mice (*Mus musculus)*, aged 3 months old, and weighing 20-40 grams (Lee's Index Value > 0.3) were randomized into three groups, namely G_1 (n = 8, control without intervention), G_2 (n = 8, moderate-intensity swimming exercise in the morning), and G_3 (n = 8, moderate-intensity swimming exercise in the night). The research was conducted at the Virology Laboratory of Veterinary Medicine Faculty within 1.5 months (28 February 2021 – 5 April 2021). The mice were placed at room temperature of 26 ± 2°C with 50-60% humidity

and the lighting was regulated by a light-dark cycle with 12-hour light and 12-hour dark cycles (08:00–20:00). The cages for the mice were 30 x 45 x 20 cm, made of plastic covered with wire gauze, and equipped with food containers and drinking bottles. Each cage contained 1 group (3–4 mice). Food and drink were given at 07.00 AM with a dose of 20 grams/mice/day. This research has followed animal welfare principles published by the European Convention for the Protection of Vertebrate Animals. All research procedures have been approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Airlangga, Surabaya with registration number: 49/EC/KEPK/FKUA/2021.

2.2 Protocol of Exercise

An acclimatization process was done before the swimming exercise intervention for 7 days. During the acclimatization process, the mice were swimming without using weights. Swimming exercises were carried out 3x/w for 4 weeks with an intensity of 6% of body weight and the duration was 70% of maximum swimming time. Morning swimming exercise was held at 08.00–09.00 AM, while night swimming exercise was held at 20.00–21.00 PM (Pranoto et al., 2020; Kwak et al., 2020). When the mice were given intervention of moderate-intensity swimming exercise in the morning and at night, the water temperature was maintained at 30 ± 2°C (Wierzba et al., 2006).

2.3 Data collection

Body weight measurements were carried out pre-training and post-training for 4 weeks using a digital harnic HL-3650 heles scale (0-5kg scale). Measurements of Lee's index pre-training and post-training for 4 weeks were carried out using the cube root formula of body weight in grams divided by naso-anal length in mm and multiplied by 10,000. Blood sampling was carried out 24 hours after the last exercise and was taken from the left ventricle of mice as much as 1-2 mL.

Then, the blood was centrifuged for 10 minutes at 3,000 rpm to separate the serum and then it was stored at –80°C for analysis of irisin levels in the next day. Irisin levels were measured using BT-Lab Enzyme-Linked Immunosorbent Assay kit BT-E1479Mo (Biossay Technology Laboratory, Inc., Shanghai, China P.R.) with standard curve range: 0.05 – 30 ng/mL and sensitivity level: 0.024 ng/mL.

2.4 Statistical Analysis

Statistical analysis was conducted using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The normality test used was the Shapiro-Wilk test, while the homogeneity test used was the Levene test. The difference test used was One-Way ANOVA, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test and linear correlation with Pearson product-moment model. All data are displayed with Mean ± Standard Error of the Mean (SEM). All statistical analyzes used a significance level ($p \leq 0.05$).

3. RESULTS

Research data including body weight data (pre-training, post-training, and delta), Lee's obesity index (pre-training, post-training, and delta) and irisin level data (post-training) are presented in **Figures 1–3**. Based on **Figure 1**, the result of one-way ANOVA test indicates that there is no significant difference in the mean weight of the pre-training mice in each group ($p \geq 0.05$), while the post-training and Δ (post–pre) show significant difference in the mean weight of mice ($p \leq 0.001$). The results of the Tukey's HSD post-hoc test shows that there is a significant difference in the mean body weight of post-training mice between G_2 and G_1 ($p \leq 0.05$), G_3 and G_1 ($p \leq 0.001$), and G_3 and G_2 ($p \leq 0.05$). Likewise, Δ (post–pre) shows a significant difference in the mean weight of mice between G_2 and G_1 ($p \leq 0.05$) and G_3 and G_1 ($p \leq 0.05$), while G_2 and G_3 do not show a significant difference ($p \geq 0.05$).

Based on **Figure 2**, the result of the one-way ANOVA test indicates that there is no significant difference in the mean Lee's obesity index of pre-training mice in each group ($p \geq 0.05$), while post-training and Δ (post–pre) mice show significant differences in Lee's obesity index ($p \leq 0.001$). The results of Tukey's HSD post-hoc test show that there is a significant difference in the Lee's obesity index of post-training mice between G_3 and G_1 ($p \leq 0.001$) and G_3 and G_2 ($p \leq 0.001$), while G_2 and G_1 do not show a significant difference ($p \geq 0.05$). The results of Δ analysis (post–pre) show that there is a significant difference in the mean Lee's obesity index between G_2 and G_1 ($p \leq 0.05$), G_3 and G_1 ($p \leq 0.001$), G_3 and G_2 ($p \leq 0.001$).

Based on **Figure 3**, the result of the one-way ANOVA test shows that there is a significant difference in the mean post-training irisin levels ($p \leq 0.001$). The results of the Tukey's HSD post-hoc test show that there is a significant difference in the mean post-training irisin levels between G_2 and G_1 ($p \leq 0.05$), G_3 and G_1 ($p \leq 0.001$), and G_3 and G_2 ($p \leq 0.05$). The results of the correlation analysis of post-training irisin levels with body weight, Δ body weight, Lee's obesity index, and Δ Lee's obesity index of post-training mice are presented in **Figure 4**.

Based on research results it was found that there was a significant negative relationship between post-training irisin levels with body weight and Lee's obesity index of post-training mice. The results of the parametric Pearson product-moment linear correlation analysis showed that post-training irisin levels were negatively correlated with body weight ($r = –0.605$, $p \leq 0.001$), Δ body weight ($r = –0.550$, $p \leq 0.001$), Lee's obesity index ($r = –0.665$, $p \leq 0.001$), and Δ Lee's obesity index ($r = –0.728$, $p \leq 0.001$).

4. DISCUSSION

The results of the analysis showed that there was a difference in the mean body weight of mice between pre-training and post-training (4 weeks) ones and they had a tendency to lose weight

in G_2 and G_3, while in G_1, the mean weight increased (Figure 1). Weight losses in G_2 and G_3 were probably due to metabolic factors and the effects of exercise (Kurdanti et al., 2015). Exercise has a positive effect in reducing body fat stores. During exercise, there is a reduction and a decrease in food intake as an energy source, which causes the increment in utilization of stored energy sources in body, especially body fat stores. Other factor that determines weight losses in G_2 and G_3 is the increased use of muscle glycogen and fat as an energy source during exercise (Ivy, 2004). If the body requires glucose in amounts greater than the amount available in glycogen, then non-carbohydrate sources such as fatty acids will be used as an energy source without needing to be converted into glucose to meet energy needs during exercise (Merawati, 2013). At G_2 and G_3, the body increased the usage of fat as an energy source if glycogen stores in the muscles were depleted (Cochran, 2010). The broken down fat comes from triacylglycerol which is stored in adipose tissue and it is the main energy store (Murray et al., 2009). During exercise, triglycerides are broken down into fatty acids to be used as energy without the need for conversion to glucose (Powers & Howley, 2015). If stored fat in adipose tissue is continuously broken down to fulfill the energy sources during exercise, and this might cause the stored fat in adipose tissue to continue to decrease, resulting in weight loss.

The analysis results showed that there was a difference between the group of the moderate-intensity swimming in the morning (G_2) and the control group (G_1) and the group of moderate-intensity swimming at night (G_3) and the control group (G_1)(Figure 3). These results are in line with the research conducted by Lu et al. (2016) which concluded that swimming exercise significantly increased irisin levels compared to the control group. Likewise, the research conducted by Kang et al. (2019) showed that swimming exercise significantly increased post-training irisin levels compared to the control group. The elevation of irisin levels in the group of moderate-intensity swimming in the morning is possibly due to exercise factor.

Exercise leads to increased activation of peroxisome proliferation-activated receptor coactivator-1α (PGC-1α) (Boström et al., 2012) to stimulate expression of fibronectin type III domain-containing protein 5 (FNDC-5) (Fatouros, 2018) and proteolytic membrane protein FNDC-5 cleavage occurs in skeletal muscle, thereby causing the release of irisin into the blood circulation (Moreno-Navarrete et al., 2013). Irisin released into the bloodstream might also be caused by an increase in energy requirements for muscle contraction during exercise, therefore stored energy in muscles decreases, which has an impact on increasing the release of irisin in bloodstream to maintain energy balance during exercise. The release of irisin in bloodstream will stimulate the browning process in white adipose tissue by inducing the expression of UCP-1 through signaling p38 mitogen-activated protein kinase (p38-MAPK) and extracellular-signal regulated kinase (ERK) (Fatouros, 2018; Perakakis et al., 2017). Irisin also increases lipolysis through the cylic adenosine monophosphate (cAMP) and protein kinase A (PKA) pathways, which leads to a decrease in fat accumulation (Perakakis et al., 2017).

The results showed that there was a significant difference in irisin levels between the moderate-intensity swimming exercise group in the morning (G_2) and the moderate-intensity swimming exercise group at night (G_3). These results are in line with the results of a research conducted by Algul et al. (2017) which concluded that night-time exercise increased irisin levels higher than morning exercise. The different results may be due to the factor of training time. Doing exercise in the morning might increase cortisol hormone, thereby suppressing the melatonin hormone receptors and having an impact on decreasing the production of melatonin hormone. The reduction production of melatonin hormone might disrupt body's metabolic processes and cause the reduction of irisin production. Doing exercise at night could reduce the production of hormone cortisol, thereby increasing the production of melatonin hormone to increase the release of irisin in bloodstream. Irisin has a circadian rhythm with the lowest level at 6:00 AM and highest level at 9:00 PM (Anastasilakis et al., 2014). However, exercise

also has a significant impact on irisin release. Exercise induces irisin release through peroxisome proliferator-activated receptor-γ (PPAR-γ) and PGC-1α (Norheim et al., 2014). PPAR-γ and PGC-1α are multispecific transcriptional coactivators which are capable in regulating several genes in response to nutritional and physiological signals in tissues. PPAR-γ and PGC-1α are expressed in skeletal muscle, brown adipose tissue, liver and heart (Gizaw et al., 2017; Norheim et al., 2014; Xu, 2013; Moreno-Navarrete et al., 2013).

Interval training increases the activation of PGC-1α, especially in heart and skeletal muscles, and increases various metabolic parameters, such as insulin sensitivity and signaling, and promotes AMPK activation as well as phosphorylation of PGC1α and FNDC5 production followed by FNDC5 cleavage to produce irisin which will be released into the blood circulation (Norheim et al., 2014; Xu, 2013; Moreno-Navarrete et al., 2013). Previous studies reported that the release of irisin in bloodstream significantly increased energy consumption and oxidative metabolism (Vaughan et al., 2015; Swick et al., 2013), which caused reduction in body fat accumulation (Perakakis et al., 2017). Thus, exercise can be used as a potential therapeutic target in the future to prevent the increasing prevalence of obesity (Anastasilakis et al., 2014).

Limitations to this current study include 1) small sample size, 2) high drop-out rate, 3) one parameter measured. Firstly, in this study, we only use a small sample size with a total sample of 24 female mice (Mus musculus). Therefore, the future study should include more female mice (Mus musculus) samples. Secondly, the high dropout rate is due to the high mortality rate of the sample during the intervention. Thirdly, we only use one parameter to measure irisin levels. Meanwhile, it required another parameter measurement, such as peroxisome proliferation-activated receptor γ coactivator-1α (PGC-1α), fibronectin type III domain-containing protein 5 (FNDC-5), markers of browning such as uncoupling protein 1 (UCP1), PR domain containing 16 (PRDM16), and other cytokines that might be related to circulating irisin levels such as interleukin-6 (IL-6).

5. CONCLUSIONS

Based on the results of this study, it was concluded that moderate-intensity swimming exercises in the morning and at night which were carried out 3x/w for 4 weeks increased irisin levels. However, moderate-intensity swimming exercise at night was more effective in increasing irisin levels of female mice (*Mus musculus*) compared to moderate-intensity swimming exercise in the morning.

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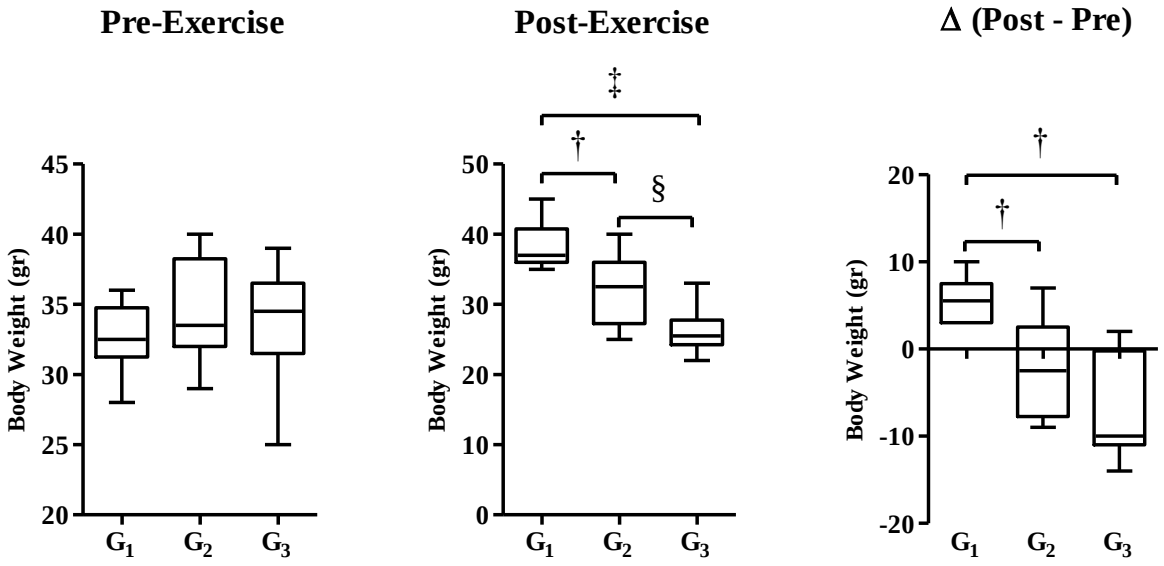

FIGURE 1. Body weight are modulated among group after exercise. Body weight data were presented as average of Mean with Standard Error of the Mean (SEM). Significant was considered with $p \leq 0.05$.

Note: G_1 (control without intervention), G_2 (moderate-intensity swimming exercise in the morning), and G_3 (moderate-intensity swimming exercise in the night). (†) significant vs G_1 ($p \leq 0.05$), (‡) significant vs G_1 ($p \leq 0.001$), and (§) significant vs G_2 ($p \leq 0.05$).

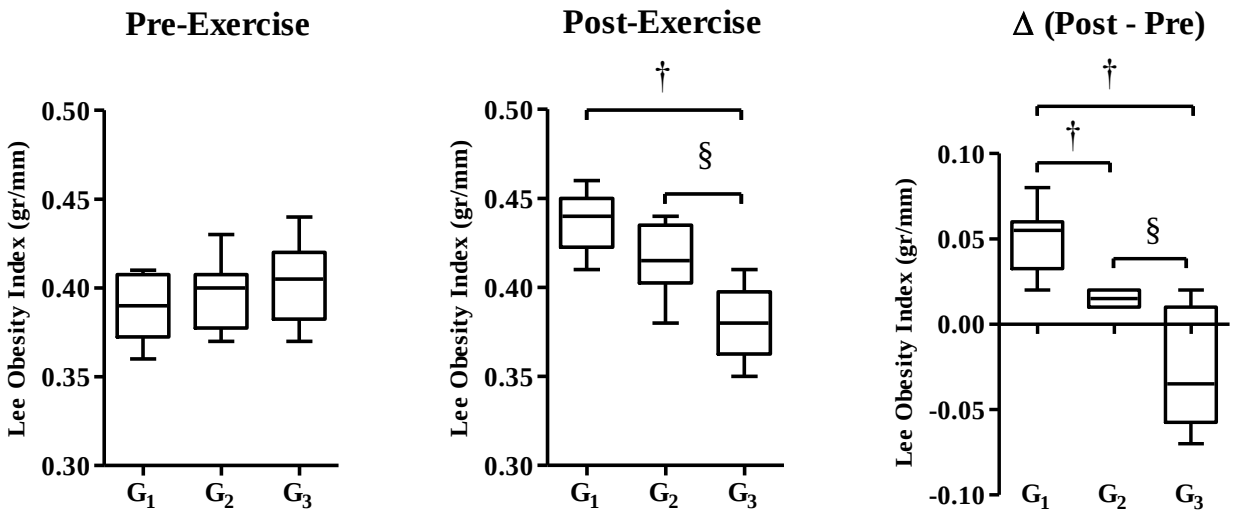

FIGURE 2. Moderate-intensity swimming exercise in the evening significantly reduced Lee's obesity index of post-exercise, and delta mice. Data were presented as average of Mean with Standard Error of the Mean (SEM). Significant was considered with $p \leq 0.05$.

Note: G_1 (control without intervention), G_2 (moderate-intensity swimming exercise in the morning), and G_3 (moderate-intensity swimming exercise in the night). (†) significant vs G_1 ($p \leq 0.05$), and (§) significant vs G_2 ($p \leq 0.05$).

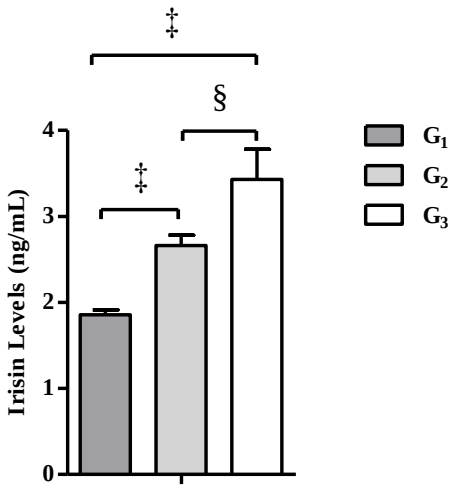

FIGURE 3. Moderate-intensity swimming exercise in the evening significantly increased post-exercise irisin levels compared to control group. Data were presented as average of Mean with Standard Error of the Mean (SEM). Significant was considered with $p \leq 0.05$.

Note: G_1 (control without intervention), G_2 (moderate-intensity swimming exercise in the morning), and G_3 (moderate-intensity swimming exercise in the night). (‡) significant vs G_1 ($p \leq 0.001$), and (§) significant vs G_2 ($p \leq 0.05$).

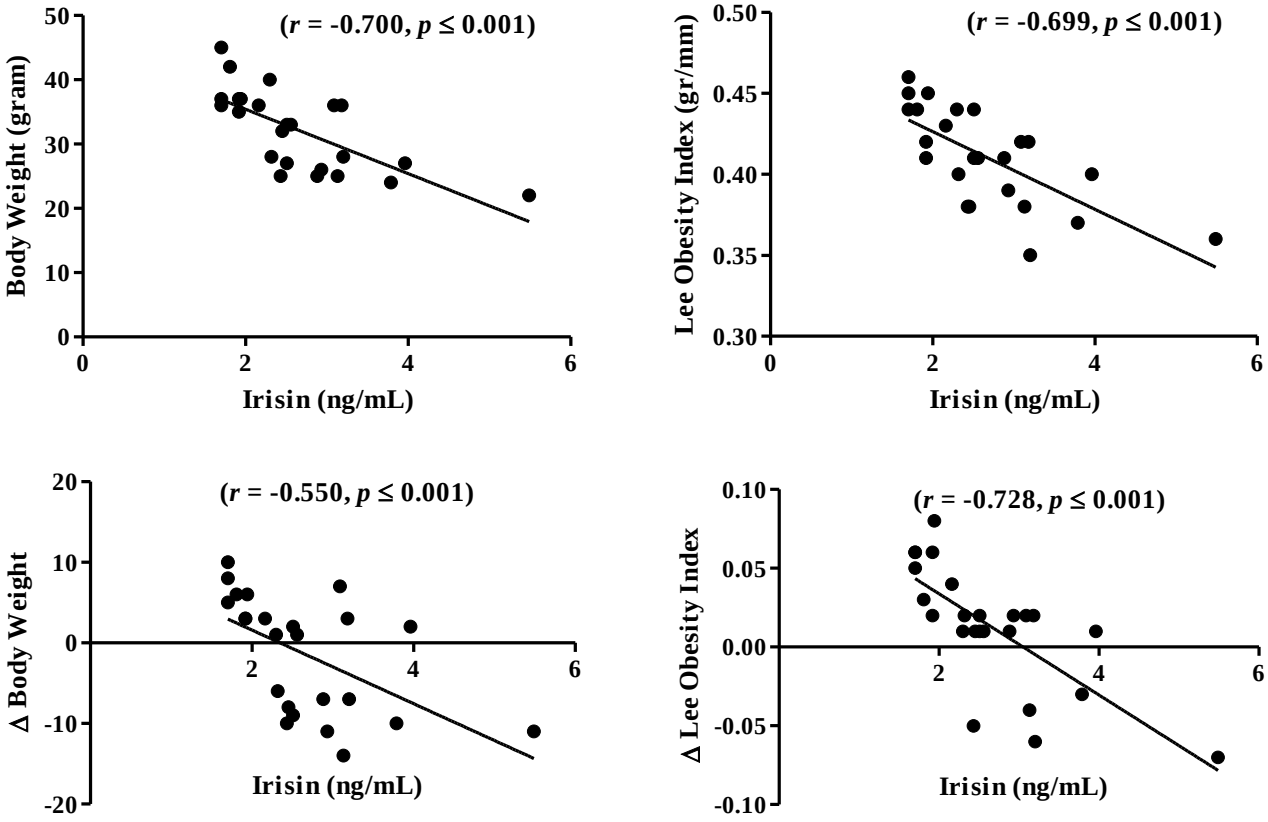

FIGURE 4. The negative correlation between irisin levels with body weight and Lee's obesity index of post-exercise mice. The significant linear correlation between parameters is visualized in the plot model ($p \leq 0.001$).

Note: Significant with $p \leq 0.001$ by Pearson's product-moment correlation test.

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Dear Dr. Korakot Nganvongpanit
Editor-in-Chief
Chiang Mai University Journal of Natural Sciences

November, 15th 2021

Thank you very much for the valuable response and suggestions from the editor and reviewers. Herewith, we resubmit the manuscript that has been revised according to the editor and reviewer comments. We look forward to the following editorial team decision. Again, thank you for your kind consideration.

Thank you for the consideration.

Sincerely,

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Dear Dr. Korakot Nganvongpanit
Editor-in-Chief
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Thank you very much for your helpful review. We have carefully studied the comments and suggestions and revised our paper accordingly. The following are our point-by-point responses to the comments. We hope that the revisions are acceptable and that our response adequately addresses the reviewer's comments. Thank you very much for your kind consideration.

Sincerely,

Purwo Sri Rejeki and coauthors

Responses to comments from Reviewer #1

Dear Reviewer. Thank you very much for your kind consideration, your valuable comments, and your comprehensive suggestions for our manuscripts. The constructive suggestions for this manuscript are crucial to improve the betterment of understanding from the global academic audiences. According to your positive comments, the brief explanation for your valuable feedback is as follow:

No	Reviewer Comments	Response
1	SUGGESTING TITLE: Nocturnal Moderate-intensity Swimming Exercise Increase more Irisin Level of Female Mice (*Mus musculus*)	▪ Thank you very much for the positive response and constructive suggestions from the reviewers. ▪ We have discussed with the team the title suggested by the reviewer "Nocturnal Moderate-intensity Swimming Exercise Increase more Irisin Level of Female Mice (Mus musculus)" we really appreciate the suggestion from the reviewer but we cannot use the title suggested by the reviewer, because in In this study, we want to show the Effect of Nocturnal and Diurnal Moderate-intensity Swimming Exercise so that we change the title to "Effect of Nocturnal and Diurnal Moderate-intensity Swimming

		Exercise on Increasing Irisin Level of Female Mice (*Mus musculus*)".
2	INTRODUCTION: Researchers hypothesized that moderate-intensity swimming exercise performed at night was more effective in increasing irisin levels of female mice (*Mus musculus*) than in the morning. --> delete this sentence.	▪ Thank you very much for the valuable suggestions related to the introduction section within the manuscript. ▪ We have revised the introduction by removing "Researchers hypothesized that moderate-intensity swimming exercise performed at night was more effective in increasing irisin levels of female mice (Mus musculus) than in the morning".
3	RESULT: Make figures less simple. please choose, positive or negative error bar caps. Below figure, explain G1=...; G2=.... etc	▪ Thank you very much for the positive response and constructive suggestions from the reviewers. ▪ We have provided an explanation of G1, G2, etc. in each figures. ▪ Note: G_1 (control without intervention), G_2 (moderate-intensity swimming exercise in the morning), and G_3 (moderate-intensity swimming exercise in the night).
4	DISCUSSION: Please state briefly about irisin circadian rhythm.	▪ Thank you very much for the positive response and constructive suggestions from the reviewers. ▪ We have revised the discussion section by briefly mentioning irisin circadian rhythm. ▪ Irisin has a circadian rhythm with the lowest level at 6:00 AM and highest level at 9:00 PM (Anastasilakis et al., (2014). The Journal of clinical endocrinology and metabolism, 99(9), 3247–3255. DOI: 10.1210/jc.2014-1367).
5	English needs improvement.	▪ Thank you very much for the positive response and constructive suggestions from the reviewers. ▪ We have corrected the English in our manuscript according to suggestions from reviewers.

Responses to comments from Reviewer #2

Dear Reviewer. Thank you very much for your kind consideration, your valuable comments, and your comprehensive suggestions for our manuscripts. The constructive suggestions for this manuscript are crucial to improve the betterment of understanding from the global academic audiences. According to your positive comments, the brief explanation for your valuable feedback is as follow:

No	Reviewer Comments	Response
1	The author must carefully explain the methods, data/results, and the discussion section. The idea of this study is quite interesting. However, the author must pay more attention to some parts of the manuscript to avoid unclear claims and judgment based on the data. Also, the author must explain briefly the limitation of this study and the novel findings as proof of new invention within this manuscript.	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ Limitations to this current study include 1) small sample size, 2) high drop-out rate, 3) one parameter measured. Firstly, in this study, we only use a small sample size with a total sample of 24 female mice (Mus musculus). Therefore, the future study should include more female mice (Mus musculus) samples. Secondly, the high dropout rate is due to the high mortality rate of the sample during the intervention. Thirdly, we only use one parameter to measure irisin levels. Meanwhile, it required another parameter measurement, such as peroxisome proliferation-activated receptor γ coactivator-1α (PGC-1α), fibronectin type III domain-containing protein 5 (FNDC-5), markers of browning such as uncoupling protein 1 (UCP1), PR domain containing 16 (PRDM16), and other cytokines that might be related to circulating irisin levels such as interleukin-6 (IL-6).

Responses to comments from Reviewer #3

Dear Reviewer. Thank you very much for your kind consideration, your valuable comments, and your comprehensive suggestions for our manuscripts. The constructive suggestions for this manuscript are crucial to improve the betterment of understanding from the global academic audiences. According to your positive comments, the brief explanation for your valuable feedback is as follow:

No	Reviewer Comments	Response
1	Blood samples were taken 12 hr after the last exercise bout in the 4-week training program. The authors are encouraged to provide information that the irisin levels were not the result of the last exercise bout.	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ We would like to clarify that the blood draw was performed 24 hours after the last exercise not 12 hours after the last exercise. ▪ Serum irisin levels will return to baseline within 24 hours (Kuloglu et al. (2014). Peptides, 55, 85–91. DOI: 10.1016/j.peptides.2014.02.008), while the level of irisin synthesis in tissues, such as skeletal muscles, liver, and kidneys will return to baseline within 6 hours (Bashar et al. (2018). Journal of basic and clinical physiology and pharmacology, 30(1), 59–71. DOI: 10.1515/jbcpp-2018- 0090). However, the research conducted by Pang et al. (2018) reported that irisin levels peaked 6 hours after exercise (20.71 ± 0.25 ng/mL) and decreased to pre-exercise levels within 24 hours (15.45 ± 0.27 ng/mL) (Pang et al. (2018). The Tohoku journal of experimental medicine, 244(2), 93–103. DOI: 10.1620/tjem .244.93). This is why we took blood samples 24 hours after the last exercise. We believe that an increase in irisin levels 24 hours after exercise may not be the result of the last exercise but may be due to adaptation to 4 weeks of exercise.
2	The irisin levels seem to be quite low compared to other studies in mice, e.g. DOI: 10.1038/boneres.2016.56 and DOI: 10.3390/nu9040410. The authors need to provide information that	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ Irisin levels in the study of Zhang et al. (2017) can be compared with our study, because the irisin levels in Zhang et al. Bone

	their irisin levels can be compared to other studies.	Research (2017) 5, 16056; DOI:10.1038/boneres.2016.56 using an ELISA kit (#EK-067-16, Phoenix Pharmaceuticals, Inc.) linear range: 6.8 – 96.1 ng/mL and sensitivity: 6.8 ng/mL, while in our study, irisin levels were measured using ELISA kit BT-E1479Mo (Biossay Technology Laboratory, Inc., Shanghai, China PR) with linear range: 0.05 – 30 ng/mL and sensitivity: 0.024 ng/mL. The difference in irisin levels may be due to differences in the linear range and sensitivity of the ELISA kit used, thus causing irisin levels in Zhang et al's study. Bone Research (2017) 5, 16056; DOI:10.1038/boneres.2016.56 has a higher value than our study. ▪ Research Lu, Y., Li, H., Shen, SW. et al. Lipids Health Disc 15, 93 (2016). DOI: 10.1186/s12944-016-0263-y who tested irisin levels using an ELISA kit (Phoenix Pharmaceuticals, Inc.; Burlingame, CA, USA) with linear range: 1.29 – 27.5 ng/mL and sensitivity: 1.29 ng/mL obtained almost the same value as our study (1.76 ± 0.17 μg/L) (1 μg/L = 1 ng/mL). Likewise the research of Tine Kartinah, et al. (2018). BioMed research international, 2018, 4708287. doi:10.1155/2018/4708287 also obtained irisin levels which were almost the same as our research. Therefore, we believe that the irisin levels in our study can be compared with the irisin levels in other studies (eg DOI: 10.1038/boneres.2016.56 and DOI: 10.3390/nu9040410) with the same linear range and sensitivity ELISA kit notes and the same sample used to perform irisin examination must also be the same.
3	It is not clear why the exercise intensity was moderate-intensity. Please clarify.	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ We wanted to find out if moderate-intensity exercise could increase irisin levels. Also,

		the long-term aim of this study is to analyze whether this model of moderate-intensity exercise can be proposed as a safety model to increase metabolism without negative impact on physiological status, especially muscle inflammation and tissue injury. Based on the previous report, Susanto et al. [International Journal of Endocrinology. (2020), Volume 2020, Article ID 3098261] mentioned that moderate-intensity exercise significantly increases metabolism and decreases levels of inflammation compared to high-intensity exercise (Paolucci et al. [Biological psychology. (2018), 133, 79–84.).
4	I suggest to change in the title "Comparison Effect of Nocturnal Diurnal Moderate-intensity Swimming Exercise in Increasing" to "Effect of Nocturnal and Diurnal Moderate-intensity Swimming Exercise on"	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ We have revised the title and welcome suggestions from reviewers "Effect of Nocturnal and Diurnal Moderate-intensity Swimming Exercise on Increasing Irisin Level of Female Mice (*Mus musculus*)".
5	Throughout the manuscript, I suggest to change "pre-exercise" and "post-exercise" by "pre-training" and "post-training".	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ Throughout the manuscript, we have revised by changing "pre-exercise" and "post-exercise" to "pre-training" and "post-training".
6	Ls13 and 90. Change "maximum time" To "maximum swimming time".	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ We have revised Ls13 and 90 by change "maximum time" to "maximum swimming time".
7	L43. Please change "$p = 0.000$." to "$p < 0.001$."	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ We have revised L43 by change "$p = 0.000$." to "$p < 0.001$."
8	L113. Data in the figures is displayed as mean and SD. Please clarify.	▪ Thank you very much for the valuable suggestions and positive response from the reviewers.

		▪ We would like to clarify that there is an error in writing the correct caption in the figure, namely the mean and Standard Error of the Mean (SEM). We have revised and changed it to mean and Standard Error of the Mean (SEM).
9	L142. I suggest to delete “Based on the results of the final study,”	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ We have removed "Based on final study results" and changed it to based on research results.
10	Ls150. Change “Discussions” to “Discussion”.	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ We have revised by Change “Discussions” to “Discussion”.
11	Ls 160-161 and Ls165. Fatty acids are available as an energy source without the need for conversion to glucose. Please revise.	▪ Thank you very much for the valuable suggestions and positive response from the reviewers. ▪ We have revised Ls 160-161 and changed it to “If the body requires glucose in amounts greater than the amount available in glycogen, then non-carbohydrate sources such as fatty acids will be used as an energy source without needing to be converted into glucose to meet energy needs during exercise”. ▪ We have revised Ls 165 and changed it to “During exercise, triglycerides are broken down into fatty acids to be used as energy without need for conversion to glucose”.