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The beneficial effects of six weeks of swimming exercise on growth hormone and cortisol levels in male mice (*Mus musculus*)

Los efectos beneficiosos de seis semanas de ejercicio de natación sobre la hormona del crecimiento y los niveles de cortisol en ratones macho (*Mus musculus*)

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Abstract. This study aimed to demonstrate the effects of moderate-intensity swimming exercise in the morning and evening over 6 weeks on the increase in growth hormone (GH) levels and the decrease in cortisol levels in male mice (*Mus musculus*). This research is a true-experimental study with a randomized control group posttest-only design. A total of 33 eight-week-old male mice (*Mus musculus*) weighing 20–40 grams were randomly divided into three groups: CN (n = 11; control without intervention), ME (n = 11; morning exercise), and AE (n = 11; afternoon exercise). Morning and evening exercises with moderate intensity were conducted for 30 minutes per session with a frequency of 3 times per week for 6 weeks. ELISA was used to evaluate posttest GH and cortisol levels in all samples. Data analysis was performed via one-way ANOVA, followed by Tukey's honestly significant difference (HSD) post hoc test, with a significance level of $p \leq 0.05$. The results revealed differences in the increase in GH levels between ME vs CN (5.71±0.88 vs 2.54±0.21 ng/mL, p=0.001), AE vs CN (7.18±1.66 vs 2.54±0.21 ng/mL, p=0.001), and AE vs ME (7.18±1.66 vs 5.71±0.88 ng/mL, p=0.009). Similarly, the decrease in cortisol levels differed among the ME vs CN (6.62±2.37 vs 10.69±4.38 ng/mL, p=0.004), AE vs CN (2.18±0.99 vs 10.69±4.38 ng/mL, p=0.001), and AE vs ME (2.18±0.99 vs 6.62±2.37 ng/mL, p=0.008) groups. This study revealed that both exercise durations significantly increased growth hormone levels and decreased cortisol levels in male mice (*Mus musculus*). However, evening exercise with moderate intensity was more effective at increasing growth hormone levels and decreasing cortisol levels than morning exercise with moderate intensity in male mice (*Mus musculus*).
Keywords: Growth hormone, cortisol, morning exercise, afternoon exercise, metabolism

Resumen. Este estudio tuvo como objetivo demostrar los efectos del ejercicio de natación de intensidad moderada por la mañana y por la noche durante 6 semanas sobre el aumento de los niveles de la hormona del crecimiento (GH) y la disminución de los niveles de cortisol en ratones macho (Mus musculus). Esta investigación es un estudio verdaderamente experimental con un diseño de prueba posterior a un grupo de control aleatorio. Un total de 33 ratones macho (Mus musculus) de ocho semanas de edad que pesaban entre 20 y 40 gramos se dividieron aleatoriamente en tres grupos: CN (n = 11; control sin intervención), ME (n = 11; ejercicio matutino) y AE. (n = 11; ejercicio vespertino). Se realizaron ejercicios matutinos y vespertinos de intensidad moderada durante 30 minutos por sesión con una frecuencia de 3 veces por semana durante 6 semanas. Se utilizó ELISA para evaluar los niveles de GH y cortisol posteriores a la prueba en todas las muestras. El análisis de los datos se realizó mediante ANOVA unidireccional, seguido de la prueba post hoc de diferencia honestamente significativa (HSD) de Tukey, con un nivel de significancia de $p \leq 0,05$. Los resultados revelaron diferencias en el aumento de los niveles de GH entre ME vs CN (5,71±0,88 vs 2,54±0,21 ng/mL, p=0,001), AE vs CN (7,18±1,66 vs 2,54±0,21 ng/mL, p=0,001) y AE vs EM (7,18±1,66 vs 5,71±0,88 ng/mL, p=0,009). De manera similar, la disminución en los niveles de cortisol difirió entre ME vs CN (6,62±2,37 vs 10,69±4,38 ng/mL, p=0,004), AE vs CN (2,18±0,99 vs 10,69±4,38 ng/mL, p=0,001), y grupos AE vs ME (2,18±0,99 vs 6,62±2,37 ng/mL, p=0,008). Este estudio reveló que ambas duraciones de ejercicio aumentaron significativamente los niveles de la hormona del crecimiento y disminuyeron los niveles de cortisol en ratones macho (Mus musculus). Sin embargo, el ejercicio nocturno de intensidad moderada fue más eficaz para aumentar los niveles de la hormona del crecimiento y disminuir los niveles de cortisol que el ejercicio matutino de intensidad moderada en ratones macho (Mus musculus).
Palabras clave: Hormona del crecimiento, cortisol, ejercicio matutino, ejercicio vespertino, metabolismo

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Introduction

Growth hormone (GH) is the primary regulator of longitudinal growth from childhood to puberty and the anabolic metabolism of mammals throughout life. It is released in a pulsatile manner, with the main secretion of GH occurring at intervals of approximately 3 hours, followed by long periods of almost undetectable basal serum GH levels in most species (Bertherat et al., 1995). The secretion rhythm of GH seems to result from the interaction of two hypothalamic neuropeptides, GH-releasing hormone (GHRH) and the inhibitory somatostatin (SOM), or somatotropin release-inhibiting factor (SRIF), at the pituitary gland and the hypothalamic arcuate nucleus (ARC). In male rodents, these two peptides are released in reciprocal cycles of 3–4 hours at the median eminence of the hypothalamus, where they enter the pituitary portal blood supply and thus reach the somatotropes in the anterior pituitary lobe (Bednarz et al., 2020). Previous studies have reported that the release of stimulating hormones from the hypothalamus, along with the cellular localization of SOM receptors in pituitary cells, reflects the secretion rhythm of pituitary hormones (Alshafie et al., 2020). Excess GH in humans and rodents, such as mice, can create a physiological state where GH receptors and prolactin receptors are simultaneously activated, while the expression of GH transgenes from mice, cattle, or sheep clarifies the specific role of GH action (Kopchick et al., 2014). Extreme increases in circulating GH also result in hyperinsulinemia despite the presence of euglycemia, which is most likely due to the diabetogenic effects of GH (Palmer et al., 2009).

Glucocorticoids, such as cortisol, are steroid hormones produced by the zona fasciculata of the adrenal cortex in the adrenal glands (Chourpiliadis & Aeddula, 2023). The presence of cortisol is considered crucial for regulating the stress response in rodents, such as mice. Stress increases the activity of the hypothalamic–pituitary–adrenal (HPA) axis and results in elevated secretion of corticosteroids from the adrenal cortex (Gong et al., 2015). Stress can activate not only the HPA axis but also other pathways within the neuroendocrine system. The activation of different neuroendocrine pathways by various stressors can differentially affect the local regulation of glucocorticoid levels in different organs, leading to varying dynamics between cortisol and corticosterone under different stress conditions (Gong et al., 2015). Previous research has reported increased cortisol levels in the plasma and adrenal glands of rats after they are subjected to stress (Won & Lin, 1995). Additionally, cortisol has much greater glucocorticoid potential than does corticosterone (Nakamura et al., 1989). Overall, the relationship between cortisol and corticosterone in rodents may differ under various physiological or stress conditions. A study by Ovejero et al. (2013) demonstrated that cortisol and corticosterone exhibit different patterns in the field and in response to acute stressors.

Stress in the body can take the form of exercise and the exercise process itself (Hackney, 2006). Specifically, for sedentary individuals, exercise can initially be a stressor, but as the body adapts, exercise transitions into eustress (Hackney & Walz, 2018). To modulate stress, this action can be beneficial and productive in the stress response through exercise. The role of cortisol postexercise has led to the development of dietary strategies aimed at suppressing cortisol levels at rest and in response to exercise. A study conducted by Viru (Viru, 1992) reported that blood cortisol levels increased proportionally with exercise intensity, with the workload at the threshold (50–60% VO_2max). In addition to cortisol, exercise can also alter GH secretion in humans and rodents. GH secretion was tested in sedentary male and female rats (without exercise) and those subjected to chronic exercise. The results revealed that sedentary male rats presented characteristic pulsatile GH secretion. Conversely, chronic exercise suppressed GH secretion in rats during exercise sessions. This study revealed that GH secretion is suppressed in response to exercise in rats, and these findings are consistent with the observed increases in human subjects postexercise (Butkus et al., 1995).

Recent studies have shown that the timing of exercise can significantly influence hormonal responses. For example, research has demonstrated that evening exercise might have a more pronounced effect on growth hormone (GH) levels due to circadian rhythms. This study demonstrated that GH concentrations rose significantly in response to exercise in the evening compared to morning sessions (Zar et al., 2021). Additionally, studies have highlighted that cortisol levels exhibit a diurnal pattern, being higher in the morning and gradually decreasing throughout the day, which can affect the overall stress response to exercise (Sabag et al., 2021; Hu et al., 2023). Understanding these temporal differences is crucial for optimizing exercise protocols aimed at hormonal regulation.

This study aimed to demonstrate the effects of moderate-intensity swimming exercise in the morning and evening over 6 weeks on the increase in growth hormone levels and the decrease in cortisol levels in male mice (*Mus musculus*). This study aims to contribute to understanding how exercise timing influences hormonal regulation, which has implications for developing more effective exercise regimens for both health and performance.

Materials and methods

The method used in this study was true-experimental with a randomized control group posttest-only design. A total of 33 eight-week-old male mice (*Mus musculus*), weighing 20–40 grams, has red eyes and white fur, and in healthy conditions (active and nondeformed) were randomly divided into three groups: CN (n = 11; control without intervention), ME (n = 11; morning exercise), and AE (n = 11; afternoon exercise). The research was conducted at the Biochemistry Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, over a period of 8 weeks (including acclimatization and intervention). The mice were housed at a room temperature of 26±2°C with 50–60% humidity and lighting regulated by a 12-hour light/12-hour dark cycle (08:00–20:00). The mouse cages measured 60 × 45 × 25 cm, were made of acrylic, were equipped with ventilation, and contained food and water bottles, with each cage housing one group (11 mice). Food was provided at 06:00 a.m at a rate of 10 grams/mice/day, while water was provided ad libitum. The food of pellet (BR-1, PT Japfa Comfeed, Indonesia Tbk) was given at 06.00 a.m. BR-1 contained 21% protein, 3-7% fat, 0.9-1.1% calcium, and 0.6-0.9 phosphor (Sari et al., 2024). During data collection, no deaths or signs of illness were found in the experimental animals, so there was no specific euthanasia action. The general health status of the animal was always evaluated during the development of an experimental protocol. All procedures applied in the study adhered to the principles of animal welfare published by the European Convention for the Protection of Vertebrate Animals and were approved by the Health Research Ethics Committee (KEPK) of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (No:71/EC/KEPK/FKUA/2022).

Morning and evening moderate-intensity exercise was conducted with a load of 6% of the total body weight (Antoni et al., 2022). Morning and evening exercises with moderate intensity were conducted for 30 minutes per session with a frequency of 3 times per week for 6 weeks. The morning exercise sessions were held from 08:00 to 09:00 AM, whereas the evening exercise sessions took place from 08:00 to 09:00 PM (Antoni et al., 2022). The exercise involved swimming the mice in a glass tank measuring 100 cm in length, 40 cm in width, and 50 cm in height, with a water depth of 30 cm. During the morning and evening exercise interventions, the water temperature was maintained at approximately 30±2ºC.

Body weight measurements were conducted via a Harnic HL-3650 digital scale (scale 0–5 kg) before and after the test over 6 weeks. Blood (1 mL) was collected from the left ventricle of each mouse. Blood collection was performed 24 hours after the last exercise session. The blood was centrifuged for 15 minutes at a speed of 3000 rpm. The serum was separated and stored at -80°C for growth hormone analysis the following day. Growth hormone levels were measured via a Mouse ELISA Kit (Cat.No. : E-EL-M0060; Elabscience Biotechnology Co. Ltd., USA), with a standard curve range of 0.31–20 ng/mL and a sensitivity of 0.19 ng/mL. Cortisol levels were measured via a Mouse ELISA Kit (Cat.No. : EA0010Ge; Biossay Technology Laboratory, Inc., Shanghai, China P.R.) with a standard curve range of 0.5--300 ng/mL and a sensitivity of 0.27 ng/mL.

Statistical analysis was performed via SPSS software version 21.0. Normality was tested via the Shapiro–Wilk test, and homogeneity was assessed via the Levene test. Differences between groups were analyzed via one-way ANOVA, followed by Tukey's honestly significant difference (HSD) post hoc test. All the data are presented as the means ± standard deviations (SDs). All the statistical analyses were conducted with a significance level of $p \leq 0.05$.

Results

The results of the 6-week study revealed changes in the average body weight between the pretest and posttest in each group, as shown in Figure 1. Moreover, the results of the analysis of the GH and cortisol levels are shown in Figures 2 and 3, respectively.

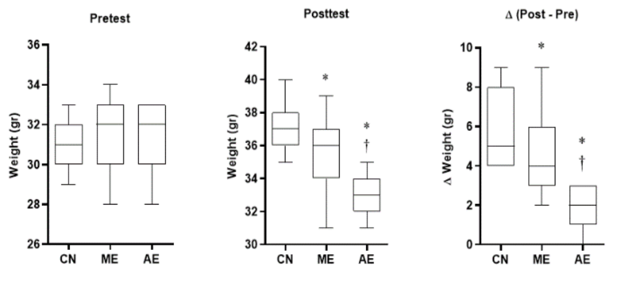

Figure 1. Pretest, Posttest, and Δ (Post–Pre) Body Weights of the Mice Description: (**) Significant vs. CN ($p \leq 0.001$). (††) significant vs. ME ($p \leq 0.01$). CN: control without intervention; ME: Morning exercise; AE: Afternoon exercise.

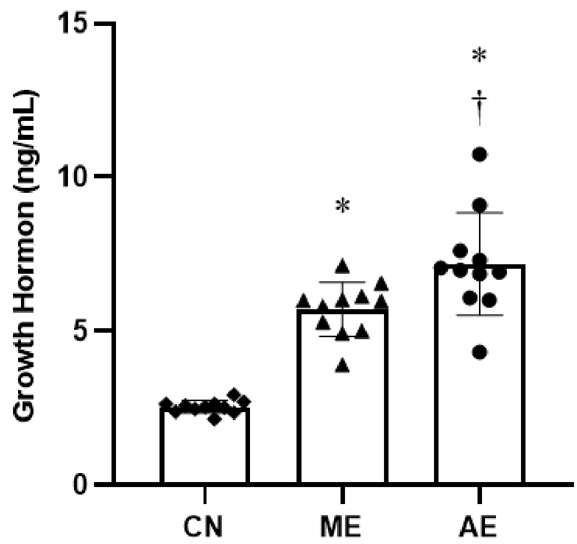

Figure 2. Growth Hormone Levels in Each Group. Description: (**) Significant vs. CN ($p \leq 0.001$). (††) significant vs. ME ($p \leq 0.01$). CN: control without intervention; ME: Morning exercise; AE: Afternoon exercise.

As shown in Figure 2, one-way ANOVA revealed the following average GH levels among the groups: CN vs ME vs AE (2.54±0.21 vs 5.71±0.88 vs 7.18±1.66 ng/mL, p=0.001). Furthermore, Tukey's honestly significant difference (HSD) post hoc test results revealed significant differences in GH levels between ME vs CN (5.71±0.88 vs 2.54±0.21 ng/mL, p=0.001), AE vs CN (7.18±1.66 vs 2.54±0.21 ng/mL, p=0.001), and AE vs ME (7.18±1.66 vs 5.71±0.88 ng/mL, p=0.009).

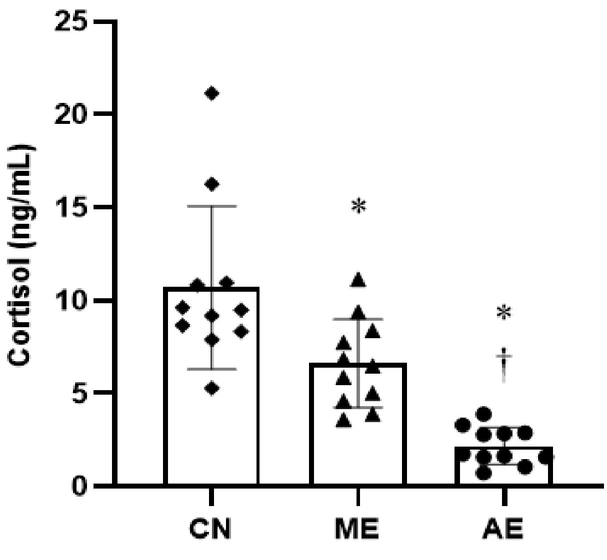

Figure 3. Cortisol levels in each group. Description: (**) Significant vs. CN ($p \leq 0.001$). (††) significant vs. ME ($p \leq 0.01$). CN: control without intervention; ME: Morning exercise; AE: Afternoon exercise.

As shown in Figure 3, one-way ANOVA revealed the following average cortisol levels among the groups: CN vs ME vs AE (10.69±4.38 vs 6.62±2.37 vs 2.18±0.99 ng/mL, p=0.001). Additionally, Tukey's honestly significant difference (HSD) post hoc test results revealed significant differences in cortisol levels between ME vs CN (6.62±2.37 vs 10.69±4.38 ng/mL, p=0.004), AE vs CN (2.18±0.99 vs 10.69±4.38 ng/mL, p=0.001), and AE vs ME (2.18±0.99 vs 6.62±2.37 ng/mL, p=0.008).

Discussion

The aim of this study was to assess the impact of moderate-intensity swimming exercise in the morning and evening on the increase in growth hormone levels and the decrease in cortisol levels in male mice. The results of this study indicate that moderate-intensity swimming exercise, whether conducted in the morning or evening, significantly increases growth hormone (GH) levels and decreases cortisol levels in male mice. These findings indicate that moderate-intensity swimming exercise, whether conducted in the morning or evening, affects the increase in growth hormone levels. However, compared with morning exercise, evening swimming exercise is more effective at increasing growth hormone levels. While these results align with previous studies, it is important to consider the limitations posed by the study's design, particularly the lack of baseline hormone measurements (Borer et al., 2024). Without initial hormone levels, it is challenging to conclusively attribute the observed changes to the exercise interventions alone (Ennour-Idrissi et al., 2015). As hormonal fluctuations naturally occur due to circadian rhythms (Shen et al., 2023), the changes seen might partly reflect these daily variations rather than solely the effects of exercise (van Kerkhof et al., 2015).

This result aligns with previous research, which reported that moderate-intensity swimming exercise over 6 weeks significantly increased growth hormone levels in male rats (Moustafa & Arisha, 2020). Additionally, Gomes et al. (2006) reported that growth hormone levels were greater after swimming exercise was performed five days per week for 6 weeks, with the aid of a weight support system comprising 5% of the body weight of the rats. Moreover, evening swimming has been reported to reduce corticosterone levels in male rats after exercise for 5 days per week over 4 weeks (Safari et al., 2020). Previous studies also reported that cortisol levels were lower in rats after 2 weeks of swimming exercise (Ibrahimaj Gashi et al., 2021). Therefore, the observed increase in growth hormone levels and decrease in cortisol levels are likely attributed to the exercise regimen.

However, given the study's design, which lacked baseline GH level data, the interpretation of the results should be approached with caution (Pranoto et al., 2024). The absence of initial hormone levels limits the ability to fully attribute the observed changes to the exercise intervention (Ennour-Idrissi et al., 2015). Furthermore, the natural circadian rhythms of GH and cortisol may have played a role in the hormonal variations observed, adding a layer of complexity to the findings (Mohd Azmi et al., 2021; Wang et al., 2021). Future studies should incorporate baseline hormone measurements to better understand the true impact of exercise timing on hormonal regulation.

Swimming exercise is one of the most commonly used moderate-intensity aerobic exercise models in animal research (Yan et al., 2022). This type of exercise can recruit a large muscle mass and induce extensive cardiovascular adaptations, including physiological cardiac hypertrophy and reduced blood pressure (Valenzuela et al., 2023). Previous studies have also reported that combining growth hormone administration with swimming exercise can influence the PI3K/AKT/mTOR signaling pathway in the left ventricular tissue of rats. However, the ERK signaling pathway was not found to be active in physiological left ventricular hypertrophy induced by swimming and growth hormone administration over 8 weeks (Palabiyik et al., 2018). Additionally, several issues related to animal experiments exist. First, the duration of swimming is commonly used as a parameter to measure exercise intensity, but the workload of swimming rodents is not uniform (Wang et al., 2020). Some animals do not exhibit continuous swimming behavior but use floating, diving, or drifting behaviors, whereas others are more active and engaged (Poole et al., 2020). Second, forced swimming exercises can induce varying levels of stress in animals, although evidence suggests that mild stress does not cause adverse effects if it is managed with appropriate procedures (Liu et al., 2018).

Exercise at an appropriate intensity serves as a strong stimulus for the secretion of growth hormone and cortisol. The pulsatile and circadian rhythms underlying these hormones can potentially modulate exercise responses. However, significant effects are observed in the cortisol response to aerobic exercise. Previous research has reported that aerobic exercise triggers cortisol hormone responses in the afternoon (Kanaley et al., 2001). A study by Moholdt et al. (2021) indicated that afternoon exercise might be more beneficial for metabolic health in overweight/obese individuals than morning exercise is because of improved glycemic control and partial reversal of metabolic profile changes induced by a high-fat diet. This finding is supported by earlier research from Kaimal et al. (2016), which reported that cortisol levels are typically highest in the morning and gradually decline throughout the day. Other studies have also shown that cortisol follows a circadian rhythm (Mohd Azmi et al., 2021). Therefore, high cortisol levels in the morning can disrupt melatonin secretion, which in turn affects growth hormone. Growth hormone is crucial in the sleep–wake cycle, and its production is regulated by melatonin. The role of melatonin in facilitating the neuroregulation of growth hormone secretion at the hypothalamic level has been demonstrated by increased growth hormone levels (Nassar et al., 2007).

In conclusion, while evening exercise appears to have a more favorable impact on GH and cortisol levels, the interpretation of these findings should be moderated by considering the influence of circadian rhythms and the lack of baseline hormone data. Future studies should aim to include baseline measurements and explore the mechanisms underlying the differential effects of exercise timing on hormonal regulation.

Conclusion

On the basis of the results of the present study, both forms of exercise significantly increase growth hormone levels and decrease cortisol levels in mice. However, moderate-intensity evening exercise is more effective than moderate-intensity morning exercise in increasing growth hormone levels and reducing cortisol levels in mice. Nonetheless, these conclusions should be interpreted cautiously due to the study's limitations, including the lack of baseline hormone measurements and the inability to control for all potential confounding variables. Future research should aim to include more comprehensive controls and baseline data to better understand the impact of exercise timing on hormonal regulation. By doing so, we can develop more precise recommendations for optimizing exercise timing to enhance metabolic and endocrine health.

Conflict of interest

The authors declare no conflict of interest in this study.

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