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Comparative Effects of Monday–Thursday and Daud Fasting Regimens on Serum Testosterone Levels in Male Wistar Rats (Rattus norvegicus)

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ABSTRACT

Introduction. Intermittent fasting has attracted increasing attention because of its metabolic and endocrine benefits. However, evidence regarding the effects of non-daily fasting regimens, particularly Monday–Thursday fasting and Daud fasting, on male reproductive hormones remains limited. This study compared the effects of these regimens on serum testosterone levels in male Wistar rats. **Methods.** A true experimental study with a post-test-only control group design was conducted using 27 healthy male Wistar rats (*Rattus norvegicus*). Animals were randomly assigned to a control group, a Monday–Thursday fasting group, or a Daud fasting group (n = 9/group). The fasting groups underwent 12-hour daily feeding restriction (18:00–06:00) for 21 days, comprising six and ten fasting days, respectively. Serum testosterone levels were measured using ELISA and analyzed by one-way ANOVA followed by Tukey's post hoc test. **Results.** Mean serum testosterone concentrations were 15.003 ± 3.55 mg/dL in the control group, 11.682 ± 3.05 mg/dL in the Monday–Thursday fasting group, and 8.501 ± 1.31 mg/dL in the Daud fasting group. Significant differences were observed among groups ($p < 0.001$). Both fasting groups showed significantly lower testosterone levels than the control group ($p = 0.049$ and $p < 0.001$), whereas no significant difference was found between the two fasting regimens ($p = 0.061$). **Conclusion.** Both Monday–Thursday fasting and Daud fasting for 21 days were associated with reduced serum testosterone levels in male Wistar rats. Further studies should control energy intake and evaluate hormonal and metabolic biomarkers to clarify the underlying mechanisms.

1. Introduction

A nutritional approach known as intermittent fasting (IF) alternates periods of eating and fasting over a specific timeframe. In recent years, this approach has been the subject of increasing research because it has been shown to provide various benefits for metabolic health, such as increasing glucose metabolism and insulin sensitivity, increasing fat oxidation, as well as reducing oxidative stress. In addition to affecting the metabolic system, intermittent fasting is also recognized for having a part in regulating various physiological systems, including the endocrine system that controls male reproductive function.[1,2]

Changes in energy balance during fasting trigger various metabolic adaptations that may potentially affect the role of the hypothalamic-pituitary-gonadal (HPG) axis. Decreased energy availability can prevent the hypothalamus from secreting gonadotropin-releasing hormone (GnRH), which in turn reduces the production of luteinizing hormone (LH) by the anterior pituitary. Reduced LH stimulation of Leydig cells in the testes can subsequently decrease testosterone synthesis. In addition to regulation via the HPG axis, changes in insulin sensitivity, increased fat oxidation, and hormonal adaptations during fasting are also thought to contribute to changes in testosterone levels.[3]

Testosterone is the primary androgen hormone that plays a vital part in sexual differentiation, spermatogenesis, the maintenance of secondary sexual traits, reproductive function, and metabolic regulation in men. Therefore, changes in testosterone levels resulting from energy restriction during fasting are of significant concern because they can affect both reproductive function and the body's metabolic adaptations. Cienfuegos et al.'s comprehensive study revealed that several types of intermittent fasting,

especially time-restricted eating, tend to lower both total and free testosterone levels in healthy young men, although the available scientific evidence remains limited and cannot yet provide consistent conclusions.[4]

Various studies on intermittent fasting generally use models such as alternate-day fasting and eating restrictions, or caloric restriction. However, intermittent fasting also includes fasting regimens that are not performed daily (non-daily fasting regimens), such as the Monday–Thursday fast and the Daud fasting. Both of these regimens have distinct biological characteristics in terms of the frequency of fasting exposure over the same time period, which theoretically could result in different metabolic adaptations and endocrine responses.[1]

Although various studies have evaluated the relationship between intermittent fasting and reproductive hormones, few studies have specifically examined the effects of non-daily fasting regimens, particularly Monday–Thursday fasting and Daud Fasting on testosterone levels using experimental animal models. Furthermore, there have been few comparative studies that have compared these two fasting regimens under identical experimental conditions. An experimental study by Kumar and Kaur showed that dietary restriction through intermittent fasting can suppress HPG axis activity, thereby lowering testosterone levels in Wistar rats.[5] However, the mechanisms of endocrine adaptation during fasting are still evolving and are thought to be influenced by the characteristics of the fasting regimen, the duration of the intervention, and the metabolic response to energy restriction.[6] Therefore, there remains a knowledge gap regarding whether differences in the frequency of fasting exposure between these two regimens result in distinct hormonal responses to testosterone regulation.

Based on the above rationale, this study aimed to compare the effects of Monday–Thursday fasting and Daud fasting for 21 days on serum testosterone levels in male Wistar rats (*Rattus norvegicus*). The findings are expected to provide experimental evidence on the testosterone response to two non-daily fasting regimens with different fasting frequencies and contribute to the existing evidence regarding the effects of intermittent fasting on male reproductive endocrine function.

Based on the above rationale, this study aimed to compare the effects of Monday–Thursday fasting and Daud fasting for 21 days on serum testosterone levels in male Wistar rats (*Rattus norvegicus*). The findings are expected to provide experimental evidence on the testosterone response to two non-daily fasting regimens with different fasting frequencies and contribute to the existing evidence regarding the effects of intermittent fasting on male reproductive endocrine function.

2. Methods

With a post-test-only control group design, this study is a real experiment with the goal of analyzing the effects of two intermittent fasting patterns Monday–Thursday fasting and daud fasting on testosterone levels in male Wistar rats (Rattus norvegicus). The study was approved by the Health Research Ethics Committee of Nahdlatul Ulama University, Surabaya (No.: 0316/EC/KEPK/UNUSA/2024) and carried out in the Animal Laboratory of the Faculty of Medicine. Twenty-seven male Wistar rats (Rattus norvegicus) weighing between 150 and 200 grams and aged between two and three months served as test subjects. Before treatment, all rats were acclimatized for seven days, and only those meeting the health criteria—as indicated by clear eyes, clean fur, good appetite, active movement, and the absence of anatomical abnormalities—were included in the study. The rats used in the study were kept in cages measuring 50 × 20 × 15 cm, with three rats per cage, and placed in the same laboratory room. Drinking water was provided ad libitum throughout the study, while daily food intake and energy intake were not quantitatively measured.

After the acclimatization period, three sets of nine mice each were created from the animals. The control group (K1) received no fasting treatment and was given a regular diet and unlimited access to water throughout this 21-day study period. The first treatment group (P1) fasted Monday through Thursday for six days during the 21-day study period, while the second treatment group (P2) underwent the Daud fasting for ten days during the same period. In both treatment groups, food was withheld for 12 hours, from 6:00 PM to 6:00 AM WIB, during which time drinking water was freely available.

Blood samples were collected via the heart (intracardiac puncture) using a sterile 5-mL syringe and then transferred into a Vacutainer tube without anticoagulant (plain tube). After allowing the blood samples to clot for 20 to 30 minutes at room temperature, the serum was extracted by centrifuging them for 5 to 15 minutes at 3000 rpm. Serum separation was performed no later than two hours after blood collection. The serum was then shipped in an ice box at a storage temperature of 2–10°C to the Satwa Sehat Indonesia Animal Clinical Research and Diagnostics Laboratory in Malang. Serum testosterone levels were measured using a competitive Rat Testosterone ELISA Enzyme-Linked Immunosorbent Assay (ELISA) Kit (BT LAB Bioassay Technology Laboratory, Cat. No.EA0023Ra) according to the manufacturer's instructions. IBM SPSS Statistics was used to examine the data. The Shapiro–Wilk test was used to assess normality, while Levene's test was used to assess homogeneity. One-Way

Table 1 Serum testosterone concentrations and statistical comparisons among study groups

Group	n	Mean ± SD (ng/mL)	Comparison with control (Tukey's post hoc)
Control (K1)	9	15.003 ± 3.55	–
Monday–Thursday fasting (P1)	9	11.682 ± 3.05	$p = 0.049$*
Daud fasting (P2)	9	8.501 ± 1.31	$p < 0.001$*

Note. One-way ANOVA: $p < 0.001$. Tukey's post hoc test showed no significant difference between the Monday–Thursday fasting and Daud fasting groups ($p = 0.061$).

Analysis of Variance (ANOVA) was used to examine differences in mean testosterone levels across groups. If statistically significant findings were found, the Least Significant Difference (LSD) test was then used. The criterion for statistical significance was established at a *p*-value of less than 0.05.

3. Results

A total of 27 male Wistar rats (Rattus norvegicus) completed the 21-day intervention and were included in the final analysis. The animals were equally allocated into three groups (n = 9/group): the control group (K1), the Monday–Thursday fasting group (P1), and the Daud fasting group (P2). The mean serum testosterone concentrations and statistical comparisons among the study groups are presented in Table 1.

The control group exhibited the highest mean serum testosterone concentration (15.003 ± 3.55 ng/mL), whereas the Daud fasting group showed the lowest concentration (8.501 ± 1.31 ng/mL). The Monday–Thursday fasting group demonstrated an intermediate mean serum testosterone concentration (11.682 ± 3.05 ng/mL), lower than that of the control group but higher than that of the Daud fasting group.

Before comparative analysis, the assumptions for parametric testing were evaluated. The Shapiro–Wilk test indicated that serum testosterone concentrations were normally distributed in all groups ($p > 0.05$), while Levene's test confirmed homogeneity of variance ($p = 0.136$). Therefore, one-way ANOVA was considered appropriate for comparing serum testosterone concentrations among the three groups.

One-way ANOVA demonstrated a statistically significant difference in serum testosterone concentrations among the study groups ($p < 0.001$). Tukey's post hoc analysis revealed significantly lower serum testosterone concentrations in both the Monday–Thursday fasting group ($p = 0.049$) and the Daud fasting group ($p < 0.001$) compared with the control group. However, no statistically significant difference was observed between the Monday–Thursday fasting and Daud fasting groups ($p = 0.061$).

4. Discussion

This study shows that two intermittent fasting regimens, Monday–Thursday fasting and Daud fasting administered over 21 days, were associated with a decrease in serum testosterone levels in male Wistar rats (Rattus norvegicus) compared to the control group. A decrease in testosterone levels was observed in both treatment groups, with the lowest average testosterone levels found in the Daud fasting group. Although the difference between the Monday–Thursday fasting and Daud fasting groups was not statistically significant, both treatment groups exhibited lower testosterone levels compared to the control group. These findings suggest that intermittent fasting regimens with a 12-hour feeding restriction period have the potential to influence hormonal regulation in the male reproductive system. These changes are thought to be related to the body's physiological response to changes in energy availability during the fasting period, which in turn affects hormonal regulation via the hypothalamic–pituitary–gonadal axis.[4,5,7]

The decrease in testosterone levels observed in this study is physiologically thought to be related to changes in energy balance that occur during the fasting period. Restricting eating times reduces energy availability, causing the body to adapt by shifting from using glucose as its primary energy source to utilizing fatty acids and ketone bodies as alternative energy sources. This metabolic adaptation is accompanied by a decrease in metabolic signals involved in regulating reproductive function, one of which is through changes in leptin levels produced by adipose tissue. Lower leptin levels can reduce stimulation of gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus, thereby decreasing GnRH secretion. This, in turn, reduces the release of luteinizing hormone (LH) by the anterior pituitary, leading to decreased stimulation of Leydig cells in the testes to produce testosterone. Thus, the energy restriction that occurs during intermittent fasting is thought to affect the function of the hypothalamic–pituitary–gonadal axis, which plays a crucial role in regulating testosterone synthesis.[4,5,7]

Although further analysis showed that the difference in testosterone levels between the Monday–Thursday fasting group and the daud fasting group was not statistically significant, the daud fasting group showed a tendency toward lower average testosterone levels. This finding suggests that a higher frequency of fasting likely imposes greater metabolic stress compared to fasting with a lower frequency. In this study, the daud fasting group underwent ten days of fasting during the intervention period, while the Monday–Thursday fasting group underwent six days of fasting. This difference in frequency is thought to increase the accumulation of energy deficit periods, causing the body to make metabolic adjustments to maintain energy

homeostasis. Under these conditions, physiological processes that are not essential for short-term survival, including reproductive function, tend to experience a decrease in activity as part of an adaptive mechanism to cope with energy constraints. However, since this study did not measure energy intake, changes in body weight, or other metabolic biomarkers, this relationship cannot yet be directly confirmed and still requires validation through further research.[8,9,10]

The results of this study are consistent with the findings of Kumar and Kaur, who reported that intermittent fasting in male Wistar rats led to a decrease in testosterone levels through disruption of the hypothalamic–pituitary–gonadal axis.[5] Similar findings were also reported by Moro et al., who showed that the implementation of time-restricted eating in healthy men over 12 months was associated with a decrease in total and free testosterone levels without a significant reduction in exercise capacity.[11] Conversely, Cienfuegos et al., in their systematic review, stated that the response of reproductive hormones to intermittent fasting still shows mixed results, as they are influenced by variations in fasting patterns, intervention duration, subject characteristics, nutritional status, and energy balance during the study.[4] These differing results indicate that the effects of intermittent fasting on testosterone levels are influenced not only by the fasting pattern employed but also by individual metabolic conditions, the duration of the intervention, and the study design used. Therefore, the results of this study reinforce the evidence that intermittent fasting patterns can affect testosterone levels, although the magnitude of the hormonal response may vary depending on the characteristics of the intervention and the study subjects.[4,5,11]

Although this study shows a decrease in testosterone levels following intermittent fasting, these findings do not necessarily indicate the occurrence of pathological reproductive dysfunction. Under conditions of energy restriction, the body undergoes various physiological adjustments to maintain homeostasis through metabolic adaptation mechanisms. One such adaptation is a temporary reduction in reproductive system activity to allocate energy toward biological processes that are more essential for survival. This response is part of the energy-conservation mechanism commonly observed during fasting periods and is expected to return to normal once energy balance is restored. Longo et al. explain that intermittent fasting induces adaptive metabolic changes, including alterations in hormonal regulation and energy substrate utilization, as part of the body's effort to maintain homeostasis.[7] Furthermore, Pecora et al. state that male reproductive function is strongly influenced by metabolic status and energy sufficiency; thus, hormonal changes during periods of energy restriction do not always reflect pathological conditions but may represent reversible physiological responses.[12] Therefore, the decrease in testosterone levels observed in this study is more appropriately interpreted as a form of physiological adaptation to energy restriction during the fasting period, although further research with longer intervention durations and measurements of other reproductive parameters is still needed to confirm its long-term effects.[7,12]

Several methodological factors should be considered when interpreting the findings of the present study. One important consideration is the possibility of an energy deficit (caloric restriction) during the fasting period. In this study, food intake and daily energy intake were not measured; therefore, it cannot be determined whether the reduction in testosterone levels resulted solely from the fasting regimen or was partly attributable to decreased total energy intake. Furthermore, changes in body weight, which may reflect metabolic status, were not evaluated, preventing direct assessment of the relationship between energy balance and testosterone concentrations. Patterson and Sears explained that the physiological responses to intermittent fasting are influenced not only by fasting patterns but also by the magnitude of energy restriction during the intervention.[13] Stratton et al. further reported that endocrine responses to fasting are determined by the interaction between fasting duration, energy balance, and individual metabolic adaptation.[8] Therefore, future studies should incorporate measurements of dietary intake, body weight, and metabolic parameters to better elucidate the mechanisms underlying testosterone changes during intermittent fasting.[8,13]

This study has several limitations that should be considered when interpreting the results. First, daily feed intake and energy intake were not measured; therefore, the contribution of caloric restriction to the decrease in testosterone levels cannot be distinguished from the effects of the fasting regimen. Second, changes in body weight during the intervention period were not evaluated; thus, the relationship between changes in metabolic status and testosterone levels could not be analyzed directly. Third, this study did not measure biomarkers involved in the regulation of the hypothalamic pituitary gonadal axis, such as leptin levels, gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), or the stress hormone corticosterone; thus, the proposed biological mechanisms are still based on evidence from previous studies. Furthermore, the light–dark cycle conditions and the timing of blood sampling were not specifically documented, even though testosterone levels are known to be influenced by circadian rhythms. Therefore, future studies are advised to control energy intake, monitor changes in body weight, document animal husbandry conditions in greater detail, and measure hormonal and metabolic

biomarkers related to reproductive function so that the mechanisms underlying changes in testosterone levels due to intermittent fasting can be explained more comprehensively.[8,11,13,14]

5. Conclusion

This study shows that the implementation of two intermittent fasting patterns, namely, Monday–Thursday fasting and Daud fasting, with a 12-hour daily eating window over 21 days, was associated with a decrease in serum testosterone levels in male Wistar rats (Rattus norvegicus) compared to the control group. The daily fasting group showed lower mean testosterone levels than the Monday-Thursday fast group, although the difference between the two treatment groups was not statistically significant. These findings suggest that intermittent fasting patterns have the potential to influence hormonal regulation in the male reproductive system through metabolic adaptations resulting from energy restriction. However, given that this study did not evaluate energy intake, body weight changes, or hormonal biomarkers involved in the hypothalamic–pituitary–gonadal axis, the mechanism underlying the decrease in testosterone levels cannot yet be directly confirmed. Therefore, further research with better control of metabolic factors and more comprehensive measurement of hormonal parameters is needed to confirm this mechanism.

6. Author Contribution

N.F.W.S contributed to the study conceptualization, research design, methodology development, supervision of the research process, validation of the findings, interpretation of the results, critical revision of the manuscript, and overall project supervision. W.W contributed to the research supervision, methodological guidance, validation of the study findings, interpretation of the results, and critical review of the manuscript. N.I.P.K contributed to the investigation, animal experimentation, data collection, data analysis, preparation of the original manuscript draft, and manuscript revision. Z.M.E contributed to manuscript editing, language editing, formatting, layout preparation, reference management, and preparation of the manuscript for journal submission. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

7. Acknowledgments

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8. References

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