JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of Time Restricted Eating On the Steroid Hormones during Fasting and in Response to an Exercise Session in Active Boys

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 16 May 2022
Accepted: 13 Jul 2022
Published: 20 Aug 2022

Keywords:
Testosterone
Cortisol
Time restricted eating
Exercise

ABSTRACT

Introduction: Nutrition programs can effectively influence physiological systems both at rest and in response to exercise. In the present study, the effect of time-restricted eating (TRE) on two steroid hormones, namely testosterone and cortisol and the ratio of testosterone to cortisol, at rest and in response to a session of exhaustive endurance exercise was investigated.

Methods: Participants of this study were 8 active boys (age: 22.63±3.50 years, body mass index: 23.46±5.61kg/m^2) who participated in the study voluntarily. Subjects performed TRE for two weeks. That is, they abstained from eating and drinking (except water) from 8 am to 4 pm, and continued their usual diet for the rest of the day. Before and after the two weeks of diet, they participated in the Yo-Yo exercise test. In both stages, blood samples were taken before the breakfast, and immediately after eating breakfast and the yo-yo test to measure the concentration of testosterone and cortisol. Paired t-tests were used to analyze the findings.

Results: Findings indicated that TRE increased fasting testosterone levels. Testosterone decreased after exercise following two weeks of TRE. Cortisol and the ratio of testosterone to cortisol at rest and in response to exercise after TRE were not significantly different compared to before TRE.

Conclusion: It can be concluded that TRE improved fasting testosterone as an anabolic index but reduced its response to exercise.

► Please cite this paper as:
Hemmat M, Dehghani A, Tinsley GM, Hemmatinafar M, Koushkie Jahromi M. The Effect of Time Restricted Eating On the Steroid Hormones during Fasting and in Response to an Exercise Session in Active Boys. J Nutr Fast Health. 2022; 10(3): 203-210. DOI: 10.22038/JNFH.2022.65581.1389.

Introduction

Testosterone and cortisol are two important steroid hormones and the end products of the hypothalamus-pituitary-gonadal and hypothalamus-pituitary-adrenal axes and their significance and physiological functions have been evaluated at rest and in response to stress/ exercise in various studies. A recent study's findings suggest that in the condition of high cortisol levels, testosterone may be neuroprotective. In contrast, low testosterone may be neuroprotective in the condition of low cortisol levels (1). High levels of testosterone and low levels of cortisol have been associated with social aggression (2). Also, the ratio of two steroids including testosterone to cortisol is known as possible biomarkers of physiological and psychological disorders (3) .

Testosterone is the strongest anabolic hormone in men which stimulates muscle growth, bone mass and androgenic potency in men. Inhibition of this hormone in men leads to a specific reduction in muscle strength and size (4). Physiological concentrations of testosterone cause a depot-specific reduction of catecholamine-stimulated lipolysis in subcutaneous fat cells, probably due to reduced expression of receptors and hormone-sensitive lipase (5). An association has been found between the low resting testosterone found in endurance-trained runners and cortisol (6). Diet and nutritional status including fasting can affect cortisol and testosterone level. A recent study indicated that resting and post-exercise cortisol increase during the first weeks of a low-carbohydrate diet. After this time, resting cortisol returns to baseline, while post-exercise

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cortisol remains elevated. High-protein diets decrease in resting total testosterone (7). Fasting could reduce stress hormones in patients with major depressive disorders (8). However, there are contradictions in some study findings which assessed the effect of fasting on cortisol and testosterone (9, 10).

Time-restricted eating (TRE) is a kind of eating pattern based on the circadian rhythm which limits duration of daily food intake (usually to ≤12 h/day), in which no obvious restriction is imposed on the quality, nor quantity, of food intake. TRE differs from other fasting protocols due to its aims on restricting eating time rather than caloric intake and can be more easily adopted by simply skipping a meal. The impact of TRE on fitness and athletic performance has been mainly related to use the Ramadan fasting protocol, which differs from TRF considering feeding schedule (11). In studies which compared TRE with usual diet, an increase in cortisol levels was observed in usual diet. Elimination of dinner has led to a significant reduction in evening cortisol at night, while non-significant increasing morning cortisol. In contrast, deleting breakfast significantly reduced morning cortisol (12).

Regarding the effect of exercise, high power resistance exercise may cause an anabolic hormonal response which partially explain the muscle hypertrophy observed in athletes who routinely employ high power resistance exercise (13). A study indicated that testosterone was elevated in the early recovery period following exhaustive endurance exercise but was reduced by 24 h after that (14). Acute response to endurance exercise stress focusing on cortisol and testosterone in professional athletes was evaluated in male professional athletes. It was found that high intensity endurance exercise induced catabolic response, but the level of response depended on a previous level of training (15).

Given the various adaptations of dietary restriction and response to short-term exercise, the aim of present study was evaluating the effect of TRE on cortisol and testosterone during fasting and in response to an exercise session.

Research Methods

The study method was a kind of quasi-experimental research with pre-test and post-test measurements. Participants of the study were 8 healthy and active young men in Shiraz whose age range was 17 to 30 years and participated in this study voluntarily and purposefully. Inclusion criteria were participating in moderate intensity exercise for at least one month, having cardiovascular health, not having a special diet in the last month, not smoking, not taking anti-anxiety and anti-depressant drugs, not taking hormonal drugs, and not suffering from obesity.

Exclusion criteria included non-adherence to the TRE plan, being affected with any metabolic or infectious diseases that affect the research variables.

Firstly, the participants were informed about the study procedures and the purpose of the study and signed the informed consent. Before participating in the research program, the subjects completed a questionnaire related to eating habits and physical activity to determine the basic information about the dietary and sports habits of the subjects. One day before the start of the research intervention program, as well as after that, the subjects' weight, height, body composition, waist and hip circumference were measured and participants were asked to avoid any changes in diet and physical activity compared to the last month, and also any dietary or energy supplements. During the TRE program, subjects were also asked to record their daily diet. The subjects were instructed to maintain their daily habits regarding physical activity, sleep and food quality and quantity during two weeks of study and their adherence to maintaining lifestyle was measured by comparing eating and physical activity before and during TRE generally and also asking the participants to report any stress or illness or any change in eating, physical activity and sleep habits within two weeks of the study. All of participants reported adhering to recommendation of maintaining lifestyle and none of them were excluded

YoYo test was used to measure cardiorespiratory fitness or VO2max of participants and also to assess its effect on testosterone and cortisol response. Validity and reliability of the test has been approved by previous studies (16). One week before taking the main test, the subjects performed the Yoyo test to be familiarized with the performance of the test and the learning effect of test can be reduced. The subjects participated in the Yo-Yo endurance exercise test twice (before and after the dietary restriction)

and between these two stages of the test, they observed TRE for two weeks. Blood samples were taken 4 times before and after TRE, during fasting and following eating breakfast and YoYo test.

Fasting blood samples were taken at 8 am, after 10 hours fasting and complete rest and sitting in a quiet situation for 30 min in the laboratory environment. Then, participants ate breakfast and 45 minutes after breakfast, subjects began warming up for 5 minutes and then performed the YoYo endurance test. Within two minutes after the YoYo test, blood samples were taken again, and the samples were sent to the laboratory in an ice chamber to assess the level of testosterone and cortisol. Subjects were then observed TRE for two weeks.

During TRE, participants were not allowed to eat any food from 8 am to 4 pm for two weeks and only drinking water was allowed. The three daily meals (50-60% carbohydrate, 25-35% fat, 10-20% protein, energy intake of 2137-2456 kcal) were designed to meet their usual energy requirements as before TRE. During the rest of the day and night, they could follow their normal diet and lifestyle and sleep pattern according to their normal routine (Jones et al., 2020).

Statistical Analysis Method

The collected data were analyzed by SPSS software version 26. Due to the normal distribution of findings approved by Shapiro-wilk test, the paired t-test was used to compare the variables in before and after TRE. Statistical significance was accepted at $p < 0.05$.

Table 1. Descriptive characteristics of participants

variables		Minimum	Maximum	Mean± Standard deviation	P value
Age(year)		17	29	22.625±3.502	-
Height (cm)		169	184	176.75±4.891	-
Weight(kg)	Before TRE	55.3	95.7	72.500±13.920	<0.001
	After TRE	54.6	94.8	71.600±13.369	
Waist circumference (cm)	Before TRE	72.8	113	88.662±14.780	<0.001
	After TRE	70	113	87.250±14.887	
Hip circumference (cm)	Before TRE	88	121.1	99.300±11.036	<0.001
	After TRE	86	117	97.887±10.425	
BMI(kg/m2)	Before TRE	17.2	33.5	23.462±5.611	<0.001
	After TRE	17.4	33.2	23.112±5.412	
VO2max (ml / kg / min)	Before TRE	20.5	39.7	31.07±7.51	0.525
	After TRE	19.3	44.5	32.37±9.32	

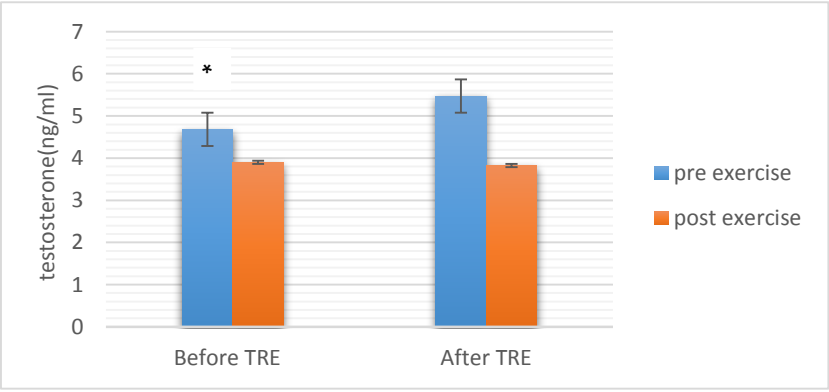

Figure 1. Comparison of testosterone before exercise (fasting) and after exercise between before and after TRE, * Significant differences with pre-exercise (pre vs post TRE), #Significant difference in testosterone post exercise (pre vs post TRE)

Results

The descriptive characteristics of the subjects are shown in Table 1 and the descriptive characteristics of the variables are shown in Table 2.

Among the variables presented in table 1, BMI, weight, and waist decreased significantly after

TRE (p<0.001), while Vo2max did not change significantly (p> 0.05).

The description and comparison of the study variables are presented in Table 2. Main aims of the study were comparison of variables before exercise and the changes (post prandial and exercise-fasting) of variables before and after TRE.

The results of comparison of fasting testosterone in before and after TRE are presented in Table 2. According to table 2, there was a significant difference between fasting testosterone levels before and after TRE (p = 0.016 and t = -.169). This means that fasting testosterone levels increased significantly after TRE compared to before TRE (Figure 1).

In order to compare the changes in testosterone after exercise, firstly the changes in testosterone in every session, including post exercise and eating breakfast –fasting state, were calculated and then the changes in each session before and after TRE were compared using paired t-test (Table 2).

As indicated in table 2, there was a significant difference in testosterone between before and after TRE (p = 0.006 and t = -3.869). This means that testosterone changed significantly following TRE compared to before TRE.

Regarding cortisol, as shown in Table 2, there was no significant difference in pre exercise cortisol between before and after TRE (p = 0.546 and t = -0.635).

In order to compare the changes in cortisol after exercise, first the changes in cortisol in every session, before and after eating breakfast were calculated and then the changes in each session (before and after TRE) were compared using paired t-test.

As indicated in table 2 there was no significant difference in post exercise changes of cortisol between pre and post TRE (p = 0.464 and t = -0.774).

Table 2. Description and comparison of variables before and following TRE

variable	Measurement time	Mean ±Standard deviation	Mean difference	t	p
testosterone ng /ml	Fasting before TRE Fasting following TRE	4.683±1.488 5.470±1.838	0.786±0.701	3.169	0.016
	Changes before TRE Changes following TRE	3.900±1.068 3.852±0.947	0.833±0.609	3.869	0.006
	Post exercise before TRE Post exercise following TRE	3.900±1.067 3.852±0.947	0.047±0.576	0.233	0.823
cortisol ng /ml	Fasting before TRE Fasting following TRE	13.837±4.592 14.600±4.737	0.762±1.397	0.635	0.546
	Changes before TRE Changes following TRE	11.712±5.997 10.587±3.197	1.887±6.895	0.774	0.464
	Post exercise before TRE Post exercise following TRE	11.712±5.997 10.587±3.197	1.125±5.173	0.550	0.599
Ratio of testosterone to cortisol	Fasting before TRE Fasting following TRE	0.448±0.404 0.441±0.271	0.033±1.515	0.063	0.951
	Changes before TRE Changes following TRE	0.399±0.155 0.377±0.084	0.570±3.552	0.454	0663
	Post exercise before TRE Post exercise following TRE	3.315±2.694 2.778±0.691	0.536±2.171	0.699	0.507

As shown in Table 2, the fasting ratio of testosterone to cortisol in before and after TRE was compared with the paired t-test. The results showed that there was no significant difference in testosterone to cortisol ratio between before and after TRE (p = 0.951 and t = 0.063).

Also as presented in table 2, the changes in testosterone to cortisol ratio between the two conditions before and after TRE were not significantly different (p = 0.663 and t = -0.454).

Discussion

The aim of the present study was to evaluate the effect of TRE on testosterone, cortisol, and testosterone to cortisol ratio during fasting and in response to an exercise session.

Fasting testosterone levels increased significantly after TRE compared to before TRE.

Also, the decreasing changes in testosterone following eating breakfast and exercise and after TRE were greater than before TRE.

Although no study was found on the subject of the present study, studies with almost similar subjects have been performed and contradictory results have been obtained. In a study, Moro et al. (2020) evaluated the effect of time TRE as limiting calories for 8 hours from 10 am to 6 pm and compared it to receiving the same calories in three meals from 7 am to 9 pm. and found that testosterone levels decreased following TRE(17). Stratton et al (2020) also found that four weeks of time-restricted feeding combined with resistance training reduced testosterone level (18). Regarding the reducing effect of fasting or TRE on testosterone, it seems that TRE could have an inhibitory effect on the Leydig cells responsible for producing testosterone. In addition, a diet with a time limit similar to calorie restriction may stimulate the AMP-activated protein kinase / acetyl-coa-carboxylase (AMPK / ACC) signaling pathway. AMPK is a central metabolic regulator that is activated during a low cellular energy state, when activated, stimulates ATP production through fatty acid oxidation and glycolysis, while simultaneously inhibiting anabolic processes. Studies in rodents have shown that short-term fasting (19-39 hours) increases AMPK and ACC in fat cells, but not in muscle. However, this hypothesis has not been confirmed in humans. In addition, calorie restriction does not appear to decrease IGF-1 concentration, although it may increase IGFBP-1(17). Malnutrition (e.g., protein restriction or protein-energy deficiency) has been suggested to impair Leydig cell function and affect testosterone biosynthesis(19). However, in the present study, testosterone increased following TRE, which indicates that TRE was not accompanied with caloric restriction or malnutrition. We wanted the participants to maintain their previous quality and quantity of diet program which may be effective on the present findings.

Considering the reduction of testosterone following eating breakfast and exercise session, it seems that eating breakfast has been effective on reducing testosterone even following exercise and this effect was more prominent following TRE. There are discrepancies about the effect of content of food on testosterone. Carbohydrate intake may affect male sex hormones. A low-carbohydrate diet (less than 5% of total energy content) decreased total plasma testosterone levels, while increased circulating levels of adrenaline, noradrenaline, and growth hormone(20). A study by Anderson et al(1987) found that a high-carbohydrate diet increased circulating testosterone and globulin-binding levels of the steroid SHBG(21), while a high-protein diet has reversed this effect. Mikolski and Zimbabwe and Nazar (2010) showed that in both low (35% protein, 64% fat, 1% carbohydrate) and high (4% protein, 1% fat, 95% carbohydrate) carbohydrate intake status, serum testosterone levels elevated in physically active people(22). However, carbohydrates appear to be positively correlated with circulating testosterone and SHBG levels in men. Increased intake of refined carbohydrates is associated with low serum SHBG levels in men and women. While according to another study no significant relationship was found between carbohydrate intake and total and free testosterone levels in healthy women (23). According to the mentioned mechanisms, it seems that TRE has probably increased testosterone by lowering blood sugar.

Regarding the reduction of testosterone following exercise and breakfast, although some studies have indicated the increasing effect of exercise on testosterone, not all sports induce the same effect. Some factors including gender, biology, and the type of exercise are effective on exercise response. Research has shown that resistance training helps short and long term increasing of testosterone levels. Resistance training such as weightlifting is the best type of exercise to boost testosterone especially in men(24) Testosterone needs many stimuli for release. The increase in testosterone due to exercise is largely influenced by the intensity, duration and type of exercise. However, debilitating activity can have a negative effect on testosterone release. Two main systems, namely the autonomic nervous system and the two axes of HPA and HPG, in response to stressful stimuli in sports, regulate the secretion of testosterone (25). Both of these systems can be influenced by blood sugar. However, in the present study, the reason for the decrease in testosterone after exercise compared to fasting could be related to food intake before exercise , as high blood sugar causes a decrease in testosterone(20). About the more decreasing effect of TRE, it is possible that this period of TRE has led to adaptations to

maintain blood sugar even under exercise conditions and may have caused maintaining blood sugar for longer duration, which is associated with a decrease in testosterone, which requires further studies in the future.

Findings of the present study indicated that there were not any significant differences in fasting and post exercise cortisol and testosterone to cortisol ratio between before and following TRE. A study by Queiroz et al (2020) found that there was an association between eating hours and fluctuations in the secretion of hormones such as cortisol(26). Solianik et al. (2020) in a study examined the effects of 48-hour fasting on several factors in elderly and obese women who were in the age range of 63 to 80 years with a body mass index of more than 25 kg / m2. Drinking water was free during this time. They found that cortisol levels increased after fasting (27).

Relevant to the present study, a review by Tinsley & La Bounty (2015) discussed studies that examined the effects of 30 or 40 hours of fasting for 3 weeks on cortisol secretion and found that cortisol did not change significantly (9). The short diet with limited time in the present study could be one of the factors affecting the lack of change in cortisol or the ratio of testosterone to cortisol in the present study. Cortisol is the final hormonal product of the hypothalamic-pituitary-adrenal axis (HPA) and plays a major role in the adaptation and regulation of homeostasis in response to internal and external challenges. As a result, cortisol mediates many metabolic processes, including increased mobility of energy substrates and increase energy delivery to the brain and muscles (28). Cortisol levels are also affected by nutrition. During the day, food intake leads to a sharp rise in salivary cortisol levels, which begins 30 minutes after the end of the meal and peaks approximately 1 hour after the start of the meal. Restricted feeding leads to increased cortisol at sunset and peaks before meals (29). The related mechanisms are not well understood, but the hypotheses that justify this phenomenon suggest that the there is an interaction between the insulin response and glucocorticoid secretion (30).

Witbracht et al. (2015) found that women who did not eat breakfast had significantly higher cortisol after meals, especially at noon(31). According to some other studies, not eating dinner led to a significant decrease in cortisol at night and a slight increase in morning cortisol, indicating that TRE increases the amplitude of the cortisol rhythm. Conversely, not eating breakfast resulted in a low daily cortisol pattern with a significant reduction in morning cortisol(32). Stratton et al. (2020) compared the effect of fasting with a typical diet pattern, both of which were 25% reduction in calories. Higher concentrations of cortisol were observed following a normal diet compared to a fasting diet (18). Similarly, another study examining the effect of an 8-week fasting diet intervention on firefighters showed that salivary cortisol levels increased in response to a fire test, decreased following diet, which may have implications for reducing the stress response (33).

In addition, the fasting can change the normal circadian rhythm increase cortisol (34, 35). However, previous research by Tinsley et al. showed no change in cortisol awakening response or change in mean cortisol concentration after eight weeks of resistance training with TRF in women (36). Discrepancies are obvious in previous study findings. The lack of change in cortisol and the ratio of testosterone to cortisol in the present study could be due to due to adaptive responses to TRE, short period of TRE, or limited number of subjects. However, maintaining the quantity and quality of eating can also limit changes in cortisol. Also, the low intensity of the exercise program can also be effective on non-significant change of cortisol following acute exercise and TRE.

The strength of the study was its novelty as well as evaluating two important hormones of testosterone and cortisol in health and exercise aspects. However, this study included some limitations. One of the limitations of present study was the wide range of BMI of participants and its possible effect on testosterone and testosterone to cortisol ratio. Another limitation of the study was the small number of participants; because of the necessity of observing time restricted eating accurately, small numbers volunteered to participate in the study which may affect the generalization of the findings.

Conclusion

In general, two-week TRE increased testosterone, which can be explained by existing background mechanisms. After eating breakfast

and exercising, testosterone decreased following TRE. Cortisol and testosterone to cortisol ratio did not change at rest and after exercise before and after TRE. In general, it can be recommended that a time restricted eating can be used to increase basal testosterone.

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