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# Acute and adaptation effect of high-intensity interval training on testosterone, cortisol and performance among collegiate running athletes



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**EDDY PURNOMO[1], NOVITA INTAN AROVAH[2], SUMARYANTO SUMARYANTO[2]**

[1] Department of Physical Education, Health and Recreation, Faculty of Sports and Health Science, Yogyakarta State University, Yogyakarta, Indonesia
[2] Department of Sports Science, Faculty of Sports and Health Science, Yogyakarta State University, Indonesia

ABSTRACT
**Purpose.** Testosterone and cortisol need to be monitored during training and recovery periods to optimise performance and avoid overtraining. This study aimed to assess the acute and adaptation effects of high-intensity interval training (HIIT) on testosterone and cortisol levels and athletic performance among collegiate athletes.
**Methods.** This research was a one-group pretest and post-test design in 20 collegiate running athletes (20.2 ± 0.7 years old) participating in a 6-week, one-hour thrice-weekly HIIT program. Outcome variables included 100-metre running performance, maximum oxygen capacity ($VO_2$max), testosterone, and cortisol serum. Rating of perceived exertion (RPE), heart rate, and blood pressure were monitored to assess the exercise intensity. Assessments were conducted before the program (T0), immediately after the first session (T1), and at the end of the 6-week HITT program (T2). The outcomes were assessed using the Friedman test. The post hoc pairwise comparisons were evaluated using the Wilcoxon signed-rank test.
**Results.** Rating of perceived exertion, heart rate, and blood pressure increased in T1 and T2, indicating that the athletes achieved high exercise intensity. Testosterone increased from T0 to T1 ($p$ = 0.009) but levelled off in T2 ($p$ = 0.668). No change in cortisol or the testosterone/cortisol ratio was demonstrated over time. Improvements in RPE ($p$ < 0.001), $VO_2$max ($p$ = 0.002), and 100 m running performance ($p$ = 0.00) were demonstrated from T1 to T2.
**Conclusions.** The program improves athletic performance, although it has limited effects on testosterone and cortisol levels. Further research using randomised control trials, larger sample sizes and extended follow-up periods is recommended to confirm these findings.
**Key words:** exercise response, exercise adaptation, testosterone, cortisol

## Introduction

Growing evidence suggests significant physiological changes in the endocrine system in response to physical exercise to maintain homeostasis and adjust to environmental demands [1]. Two hormones closely regulated during and immediately after exercise are testosterone and cortisol. Testosterone, an anabolic hormone produced by Leydig cells in the testicles, involves various physiological functions [2]. It intervenes in protein synthesis in the muscles, bone remodelling, and erythropoiesis [3]. It also increases the oxidation of lactate to generate energy during exercise by regulating the function of lactate transport proteins [4]. On the other hand, cortisol is a catabolic hormone produced by the adrenal glands that promotes energy substrate mobilisation [5]. Cortisol is modulated by circadian rhythms, but mental stress, dehydration or food can also alter its production [1]. Both hormones play a major role in physiological adaptation to exercise, mainly for maintaining positive energy status, anabolic/catabolic balance, and body composition [1], to optimise performance and avoid overtraining. A decrease in the testosterone and cortisol (T/C) ratios, in particular, suggests potential disorders before clinical symptoms (e.g., overtraining, anxiety, and depression) may appear. In contrast, an increase in the T/C ratio is indicative of a successful exercise adaptation [6] and has been used as a performance index for athletes [7].

A meta-analysis recently reported an increase in

*Correspondence address:* Novita Intan Arovah, Department of Sports Science, Faculty of Sports and Health Science, Yogyakarta State University, Colombo Street No 1, Yogyakarta, Indonesia, 55281, Indonesia, e-mail: novita@uny.ac.id, https://orcid.org/0000-0002-0779-3344

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testosterone and cortisol following exercise [8]. However, the available evidence in this field also suggests that the acute effect of exercise on testosterone and cortisol levels may vary between exercise protocols [9]. Protocols that are high in volume, with moderate to high intensity, with short recovery intervals and stress in a large muscle mass are more likely to produce the most testosterone [10] and cortisol [10, 11] compared to low-volume, high-intensity protocols using long recovery intervals. A meta-analysis also found significant differences in the standardised effect sizes of aerobic, resistance, and power exercise on salivary testosterone and cortisol [12]. In addition to the intensity, duration, and type of exercise, the fitness status of individuals is also a factor with a significant impact on these hormones' response to exercise. Thus, the effect of a specific type of exercise on trained or untrained individuals may differ [8].

Among several methods that can be used to prescribe exercise intensity are the % of heart rate reserve (%HRR) [13] and the rating of perceived exertion (RPE) scale [14]. Studies suggest that a %HRR of 60 to 90% [13] or an RPE of at least 17 indicates high-intensity exercise [14]. Those parameters can be used to design High-Intensity Interval Training (HIIT), a type of exercise involving repeated, brief to long bouts of high-intensity exercise interspersed with light-intensity exercise or rest [15, 16]. High-Intensity Interval Training has become a primary focus of research in sports science due to its role in triggering adaptations in both central (i.e., cardiovascular) and peripheral (i.e., skeletal muscle) components linked to improvement in health and sports performance [16]. The exercise can also promote the development of the fast-twitch fibres that are of utmost importance for non-endurance athletes [17], such as athletes in 100-metre sprints.

A meta-analysis of the acute effect of a single HIIT session on testosterone and cortisol levels has indicated that both hormones increase immediately after a single HIIT session, then drop below baseline levels, and finally return to baseline values after 24h [8]. However, to date, the acute effect of HIIT on testosterone and cortisol levels in trained individuals remains unclear. Also lacking is the exploration of the effect of HIIT interventions or programs, which are composed of several acute bouts of HIIT on testosterone and cortisol regulation. Additionally, although several studies have been conducted to study the acute effect of exercise programs on testosterone and cortisol levels, previous studies have mostly focused on salivary samples [18-20]. While salivary hormone is considered to reflect free, unbound hormones, several issues may limit the value of integrating salivary hormones because the levels can be substantially influenced by the sample collection methods and are sensitive to storage conditions when the samples have been archived [21]. Moreover, a study reported a lack of correlation between serum and saliva testosterone, suggesting that salivary testosterone may not be an appropriate method to examine changes in testosterone. Most previous studies also explored the effect of exercise on testosterone and cortisol levels among untrained individuals [12, 22–24], while studies into the acute and adaptation effects of HIIT programs on testosterone and cortisol levels among trained athletes are still scarce. In response, this study aimed to assess the acute and adaptation effects of a HIIT program on serum testosterone and cortisol levels and athletic performance among male collegiate athletes. The intensity of the program was based on %HRR and RPE. The effect of the program on the 100-metre sprint performance was also measured. The information is required for exercise clinicians and coaches to prescribe appropriate exercise programs to enhance the health and performance of athletes.

## Material and methods

### Research design and participants

This research was a one-group longitudinal study on 20 male collegiate running athletes in the Faculty of Sports Science, Yogyakarta State University in Yogyakarta, Indonesia.

### High-Intensity Interval Training protocols

The athletes participated in a 6-week HIIT program, which was conducted 3 days a week for around 60 to 75 minutes per session. There were four sets of HIIT in each session. Each set consisted of a regular run for 1 minute with a 70–75% heart rate reserve (HRR), a 30-second sprint to achieve 85–90% HRR, and a regular run again for 1 minute without rest and carried out for 12 to 15 minutes. The participants had no other training program and were required to refrain from moderate to vigorous physical activity outside the program.

### Outcome variables, instruments, and assessment schedules

The main outcome variables were the 100-metre running performance, maximum oxygen capacity ($VO_2$max) and the testosterone and cortisol serum.

The additional outcome variables were the rating of perceived exertion, heart rate, and blood pressure to monitor the exercise intensity. Participants' characteristics, including age, height (in metres), and weight (in kilograms), were also assessed at the beginning of the program.

The tests were carried out in the evening between 4:30 and 6:00 p.m., mainly to minimise the diurnal variation of cortisol levels. The pretest (T0) was performed immediately before the first exercise session. The acute effect (T1) was measured immediately after the first exercise session. The adaptation effect (T2) was measured immediately after the last session of the 6-week HIIT program.

*Main outcome variables*

The 100-metre running assessment was conducted on a 100 m track. The test was preceded by a short standardised warm-up for sprints. The starting position was standardised, with a stationary position with a foot behind the starting line, with no rocking movements. The test involved running a single maximum sprint over a 100-metre distance, with the time recorded.

The $VO_2$max was assessed with the 1.5-mile test [25]. The test was developed on college-age males and females. The time in minutes required to cover the 1.5 miles was recorded in this test. The $VO_2$max was computed with the following equation:

$$VO_2\text{max (ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = 88.02 + (3.716 \cdot \text{sex}) - (0.0753 \cdot \text{body weight in pounds}) - (2.767 \cdot \text{time for 1.5 miles in minutes})$$

The sex code for males was 1, and for females was 0. The developer reported a high validity of prediction ($R^2$) of 0.90 and a standard error estimate (SEE) of 2.8 ml · $kg^{-1}$ · $min^{-1}$ [25].

The blood drawing and the analysis of the testosterone and cortisol were conducted by phlebotomists and laboratory technicians from a private laboratory in Yogyakarta. Blood samples were collected through an indwelling venous catheter inserted into the cubital vein, and 5 mL of blood was drawn for each sample. The blood samples were immediately centrifuged at 3000 rpm at 4°C for 20 minutes. The serum was then separated and stored at –80°C until assayed. Serum cortisol levels were determined by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The intra- and inter-test coefficients of variation for this test were 3.2% and 6.8%, respectively. The serum testosterone concentrations were determined by ELISA using a commercial DSL kit (Laboratory Diagnostic Systems, Webster, TX). The intra-assay coefficient of variation for this assay was 4.8–5.3%, the inter-assay CV was 2.8–4.9%, and the sensitivity was 0.04 ng/ml.

*Additional outcome variables*

The Rating of Perceived Exertion was measured using the Borg Rating Scale [14]. The Borg Rating Scale is a tool for measuring an individual's effort and exertion, breathlessness, and fatigue during physical work. The participants were asked to rate their exertion on the scale during the activity, combining all sensations and feelings of physical stress and fatigue as a whole feeling of exertion. The response option ranged from 6, as no exertion at all, to 20, which reflects maximal exertion. A rating of RPE of at least 17 indicates high-intensity exercise. The ranges have been found to have a high correlation between the scale and heart rate [14].

Heart rates and blood pressure were taken by an experienced nurse. The heart rates were measured by palpating the radial artery for 15 seconds. The number was multiplied by four to calculate the beats per minute. The blood pressure was measured using the Nova-Presameter Riester sphygmomanometer (Germany) with the auscultatory method in the seated position.

*Participant characteristics*

Participants' characteristics included age, weight, and height. The research assistants measured the weight to the nearest 0.1 kg with the Omron HBF-375 scale and height to the nearest 0.1 cm with a portable Seca 213 stadiometer. Participants were barefoot and wore light clothing. Body mass index (BMI) was calculated by dividing weight (kg) by height (m) squared.

Statistical analysis

Descriptive statistics were calculated to summarise body weight, height, body mass index, resting heart rate, and blood pressure. Descriptive data are presented as mean ± standard deviation. After testing for the normality of the data, comparisons between variables at T0, T1 and T2 were performed using the Friedman test for accounting the non-normally distributed data. The pairwise post hoc comparisons were then conducted using the Wilcoxon signed-rank test. All statistical analyses were calculated using SPSS 25 at a significance level of 0.05.

**Ethical approval**

The research related to human use has complied with all the relevant national regulations and institutional policies, has followed the tenets of the Declaration of Helsinki, and has been approved by the Human Research Ethical Committee of the Universitas Gadjah Mada, Indonesia (number KE/0756/06/2021).

**Informed consent**

Informed consent has been obtained from all individuals included in this study.

## Results

Participant characteristics

Table 1 illustrates the height, weight, body mass index, and age of the participants. As seen in the table, the participants were relatively lean, and none was considered overweight or obese based on the body mass index. Participants' age range was 20 to 23, thus consistent with typical collegiate athletes' age range. No outliers or extreme values were found in these participants' characteristics.

Table 1. Participant Characteristics

| Variable | Mean | *SD* | Minimum | Maximum |
|---|---|---|---|---|
| Height (cm) | 169.4 | 4.8 | 158.0 | 175.0 |
| Weight (kg) | 58.4 | 6.1 | 49.0 | 71.5 |
| Body mass index | 20.3 | 1.8 | 17.4 | 24.7 |
| Age (year) | 20.2 | 0.7 | 20.0 | 23.0 |

Acute and adaptation effect of the exercise program on the outcome variables

Table 2 compares the outcome variable before the exercise program (T0), immediately after the first exercise session (T2) and immediately after the last session of the 6-week exercise program (T2). The ratings of perceived exertion, heart rate, and blood pressure significantly increased after the exercise session. These increases suggest that the program's intensity achieved the target exercise intensity. Decreases in the 100 m running performance and 1.5-mile running time were reflected in the reduction of the $VO_2$max. However, both the 100 metre and 1.5-mile running performance improved in the last session, suggesting the positive adaptation effect of the HIIT program on the running performance. The HIIT did not affect the anabolic and catabolic parameters except for testosterone, which improved immediately after the first exercise session (T1). The response, however, appeared to level off in T2 compared to T0 ($p$ = 0.668).

Figure 1 further illustrates the range of testosterone and cortisol and the ratio of testosterone and cortisol levels among T0, T1 and T2.

As illustrated in Figure 1, there was an acute increase in testosterone (T0–T1) among the participants, but there were minimal long-term (adaptation) response changes (T1–T2), due to the 6-week HIIT training.

## Discussion

This study explored the acute and adaptation response of high-intensity interval training (HIIT) on testosterone and cortisol levels and athletic performance among collegiate athletes. We found an increase

Table 2. Acute and adaptation effect of the exercise program on the outcome variable

| Variable | T0 Mean ± *SD* | T1 Mean ± *SD* | T2 Mean ± *SD* | *p* | T0–T1 | T0–T2 | T1–T2 |
|---|---|---|---|---|---|---|---|
| 100 m (s) | 13.3 ± 1.0 | 14.1 ± 1.4 | 12.7 ± 0.8 | 0.000 | 0.000 | 0.000 | 0.000 |
| 1.5 miles (s) | 729.7 ± 83.7 | 741.2 ± 153.4 | 669.1 ± 82.6 | 0.000 | 0.002 | 0.000 | 0.002 |
| $VO_2$max | 48.4 ± 4.1 | 47.9 ± 7.3 | 51.2 ± 4.1 | 0.000 | 0.002 | 0.000 | 0.002 |
| Testosterone | 4.6 ± 1.5 | 5.2 ± 1.7 | 5.1 ± 1.3 | 0.046 | 0.018 | 0.091 | 0.668 |
| Cortisol | 12.4 ± 3.7 | 12.4 ± 3.0 | 13.8 ± 3.0 | 0.538 | 0.629 | 0.052 | 0.108 |
| T/C | 0.4 ± 0.2 | 0.5 ± 0.2 | 0.4 ± 0.1 | 0.086 | 0.117 | 0.970 | 0.086 |
| RPE | 6.0 ± 0.0 | 17.4 ± 0.8 | 15.6 ± 1.1 | 0.000 | 0.000 | 0.000 | 0.000 |
| Heart rate | 65.7 ± 7.0 | 167.2 ± 5.1 | 149.1 ± 3.6 | 0.000 | 0.000 | 0.000 | 0.000 |
| Systole | 130.9 ± 8.6 | 164.4 ± 5.7 | 165.2 ± 6.5 | 0.000 | 0.000 | 0.000 | 0.779 |
| Diastole | 79.6 ± 6.1 | 89.0 ± 6.0 | 87.8 ± 13.4 | 0.027 | 0.001 | 0.029 | 1.000 |

T0 – baseline, T1 – immediately after the first exercise session, T2 – immediately after the 6-week HIIT program
T – testosterone, C – cortisol, RPE – rating of perceived exertion

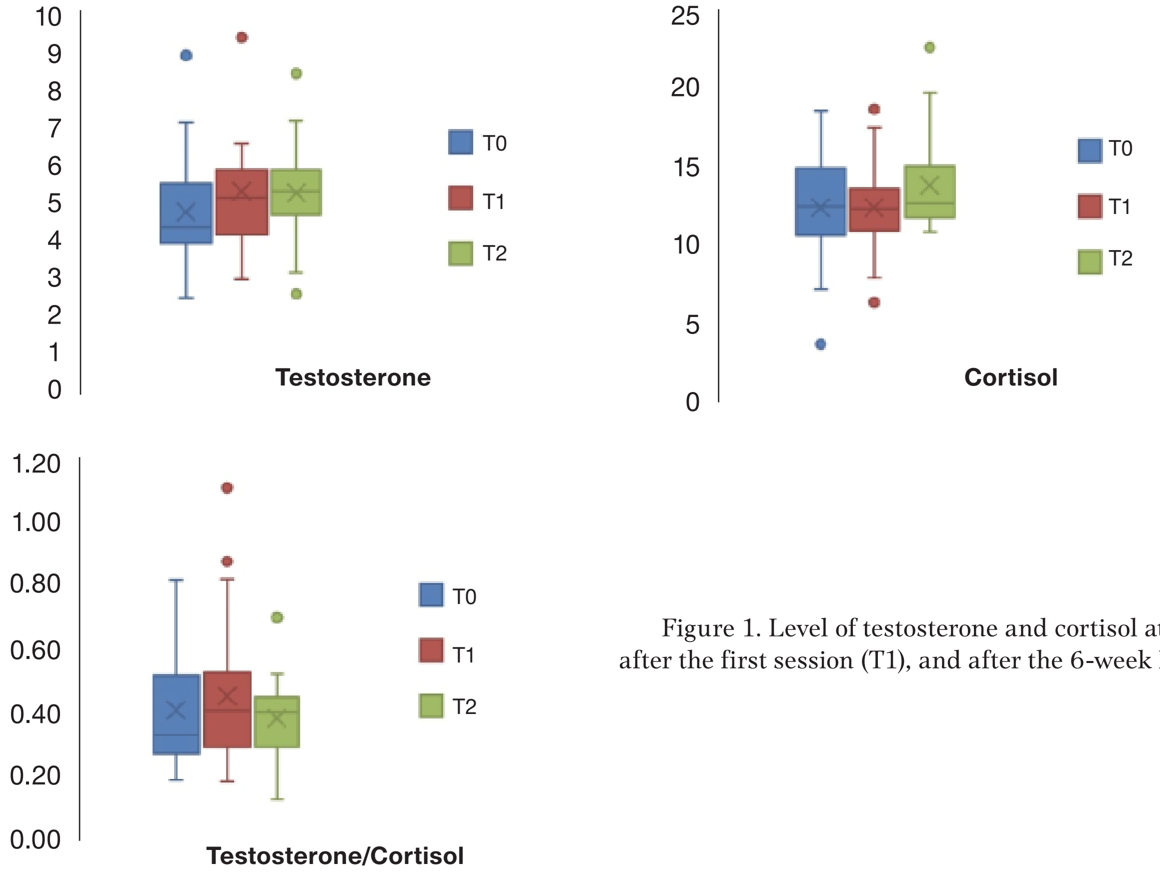


Figure 1. Level of testosterone and cortisol at baseline (T0), after the first session (T1), and after the 6-week HIIT program (T2)

in testosterone from T0 (before the first exercise session) to T1 (immediately after the first exercise session). However, the testosterone level levelled off at the end of the 6-week HIIT training (T2). We did not find changes in cortisol levels across the observation, and while there was an increase in testosterone from T0 to T1, the change in the testosterone/cortisol ratio was not demonstrated over time. However, we found increases in $VO_2$ max and 100 m running performance from T1 to T2 and a tendency to improved recovery time, as indicated by decreases in RPE and heart rate from T1 and T2.

The findings, to some extent, are inconsistent with finding from a study on untrained men aged 35 to 40 years old participating in an 8-week high-intensity exercise program combined with strength circuit training [26]. The authors reported statistically significant increases in serum testosterone (by 36%), the ratio of testosterone and cortisol (by 59%), and a reduction of cortisol (by 12%) at the end of the program compared to baseline. In contrast, the authors did not report a significant $VO_2$ max improvement in their participants after the program. Another study exploring the effect of four weeks of moderate-intensity exercise significantly increased the salivary cortisol and testosterone levels, along with a significant decrease in the ratios between the testosterone and cortisol levels of young, healthy volunteers (15 to 25 years old) [27]. Similarly, a 12-week aerobic exercise intervention has been reported to increase the serum total testosterone, free testosterone, and bioavailable testosterone levels in overweight/obese men [28]. The discrepancies may arise from the differences in the exercise protocol and the characteristics of the subjects.

Age, in particular, may play a significant role in the testosterone and cortisol metabolism. A study comparing the serum testosterone and cortisol between boys and men reported that men had higher testosterone levels than boys in the baseline. In comparison, the cortisol levels in men were higher at baseline and post-exercise [29]. The authors also reported a reduction in serum testosterone and an increase in cortisol post-exercise in both boys and men [29]. It should be noted that the authors conducted the experiment in the morning with a fasting state. Their exercise protocol was a swim-bench maximal strength task, a 200-m swim sprint followed by a high-intensity interval swimming protocol [29]. Another study in prepubertal boys resulted in an increase in testosterone and a reduction in cortisol post-exercise in both the resistance and plyometric exercise protocols conducted in the evening [18]. This study suggests that in young male athletes, multiple modes of exercise can lead to a transient anabolic state for maximising growth and development when exercise is performed in the evening. Meanwhile, an increase in cortisol and a decrease in testosterone were evident in athletes after competing in a 5000 m race [7]. The age differences, time of assessment and the exercise protocol among these studies and this present study may contribute to the discrepancies in the findings.

In contrast to previous studies, we only found a significant increase in testosterone that appears to level off at the end of the training program and no significant changes in the cortisol and testosterone/cortisol ratio throughout the program. At the same time, however, improvements in the RPE, heart rate, and athletic performance were demonstrated at the end of the program. The improvement in the RPE and heart rate from T1 to T2 suggested subjective and objective fatigue adaptation of the HIIT program. The exercise intensity or the period of the HIIT program, however, may not be sufficient to elicit testosterone and cortisol adaptation. The effect sizes of the HIIT program on the testosterone and cortisol levels may also require larger sample sizes to detect changes in this hormonal adaptation. Further research with a more extended period, more intensive HIIT program and larger sample size is thus required to confirm these findings.

Finally, the findings of this study and previous studies emphasise the challenge in interpreting the data collected from the athletes due to the complex interaction between the hypothalamic-pituitary-gonadal axis in regulating testosterone, and the hypothalamic-pituitary-adrenal axis in regulating cortisol, and may be different under different exercise protocols and individuals' characteristics. Nevertheless, this present study emphasises the feasibility of the HIIT program in improving athletic performance based on the use of the testosterone/cortisol ratio in the evaluation of the training. The findings of this study, therefore, extend the use of the 6-week HIIT program in improving athletic performance among male collegiate running athletes.

Several limitations of this study, however, need to be acknowledged. First is the lack of a control group in this study to confirm the results are due to the HIIT program rather than extraneous variables. We, however, required participants to refrain from moderate to vigorous physical activity outside the program. Secondly, our participants were homogeneous; thus, the findings from this study have limited generalisability to other populations. Third, due to resource constraints, our sample size may not have been adequate to detect changes in testosterone and cortisol adaptation due to the HIIT program. The findings of this study, however, can be used as the basis for conducting an apriori power analysis to determine an adequate sample size for future studies. Further research, including randomised control trials, is also recommended to confirm the findings of the study.

## Conclusions

An acute increase in testosterone after a HIIT session is demonstrated. However, the response appeared to level off after the 6-week HIIT program among collegiate athletes. In contrast, no change in the cortisol or testosterone/cortisol ratio was demonstrated over time. The exercise program also improved the athletic performance (i.e., 100 running time and $VO_2$ max) and recovery (i.e., post-exercise RPE and heart rate), suggesting the benefit of the HIIT program, despite the lack of an effect on the testosterone and cortisol levels. Further research, however, is required in a more heterogeneous population, larger sample size and a longer follow-up period to confirm these findings.

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## Disclosure statement

No author has any financial interest or received any financial benefit from this research.

## Conflict of interest

The authors state no conflict of interest.

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