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RESPONSES OF SALIVA TESTOSTERONE, CORTISOL, AND TESTOSTERONE-TO-CORTISOL RATIO TO A TRIATHLON IN YOUNG AND MIDDLE-AGED MALES

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Abstract. Saliva testosterone and cortisol have been suggested to represent the biologically active forms of the respective hormones. This study investigated the acute responses of saliva testosterone, cortisol, and testosterone-to-cortisol ratio (T/C) after a triathlon in young and middle-aged recreationally-trained males. Thirty-five subjects were divided into the Young (18-35 years, n=12) or Middle-aged (MA, 40-56 years, n=23) group. Pre- and post-race saliva testosterone and cortisol concentrations were measured by radioimmunoassays. Baseline testosterone was lower in MA, compared to the Young group. Cortisol and T/C decreased significantly after the race in both groups. Testosterone increased after the race in MA. Race-induced change in T/C was correlated with age ($r=-0.686$, $p<0.05$) in the Young group and change in cortisol in both groups. ($r=-0.729$, $p<0.01$ in Young; $r=-0.714$, $p<0.01$ in MA). Race-induced change in testosterone was correlated with age ($r=0.487$, $p<0.05$). This study suggested that the decline in T/C after intensive endurance exercise may be more significant with advancing age until mid-30 s, then it may reach the plateau after the fourth decade of life. The age-related decline in testosterone may be temporarily normalized after intensive endurance exercise in middle-aged men. Race-induced change in cortisol was the major determinant of that in T/C.

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Key words: Testosterone – Cortisol – Triathlon – Age – Recreationally-trained

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Introduction

Cotisol has been used as an indicator of catabolic state for its role in gluconeogenesis via the proteolytic pathway. On the other hand, testosterone has been viewed as anabolic indicator as it can stimulate glycogen storage and muscular protein synthesis [18,19,27]. An equilibrium between anabolic and catabolic states in athletes is often represented by the ratio of these two hormones, the testosterone-to-cortisol ratio (T/C) [2,3,5,38]. T/C has been shown to be decreased after intensive endurance exercise [14,23] and chronic high volumes of endurance training [33,37-39].

It has been demonstrated that salivary testosterone and cortisol was a better measure of the biologically active fractions of these hormones than those obtained from serum [26]. In addition, salivary testosterone revealed the decline in testicular function associated with aging [17,29]. Only the free fraction of these hormones in plasma can move across cell membranes and elicit biological responses. More than 95% of the testosterone in plasma is bound to sex-hormone binding globulin and albumin [35], while approximately 80% of the plasma cortisol is bound to corticosteroid binding globulin and albumin [4]. Furthermore, saliva collection is fast, noninvasive, and may reduce the stress response of cortisol during the sampling process. Thus, monitoring changes in saliva has been successfully used in investigating the responses of testosterone and cortisol during various competition and training periods [8,9,22,27].

Over the past two decades, endurance exercise such as long distance swimming and running has become one of the most popular forms of physical activity, with increasing number of middle-aged participants. Although the benefits of this type of exercise on the cardiovascular system and controls of diabetes and obesity have been well-established [1,25,30], the information regarding the age effect on acute hormonal responses and balance between anabolic and catabolic states after intensive endurance exercises is still lacking. The aims of this study were to investigate the acute changes in saliva testosterone, cortisol, and T/C levels after a triathlon in young and middle-aged men.

Materials and Methods

Subjects: Thirty-five male subjects who participated in the 2nd President Cup triathlon in Taiwan (1.5 km swimming, 40 km bike course, 10 km running) were recruited for this study. All subjects signed the informed consent. The subjects were categorized into the Young (18-35 years, n=12) or Middle-aged (MA, 40-56 years, n=23) group.

The training experience and protocols were obtained in personal interviews on the day prior to the race. The subjects have trained recreationally for 2 to 23 (median 14) years. During the 2 months prior to the race, the subjects had weekly training time of 10 to 21 (median 16) hours, containing 2-9 km of swimming, 40-160 km of cycling, and 10-120 km of running.

All subjects awaked between 5:00 to 6:00 in the morning on the race day. The outdoor temperature was 19°C at the start of the race at 9 am. All subjects gave written and informed consent. The study protocol was approved by Human Subject Committee of Fooyin University. All the sampling procedures were in accordance with the ethical standards of the Helsinki Declaration of 1977, as revised in 1983.

Saliva collection: The saliva samples were collected 1-1.5 hours before the race and within 5 min after the finish by spitting through straws into screw-capped 15 ml centrifuge tubes. The baseline saliva samples of all subjects were collected within a 20-min period. All post-race saliva samples were collected within a 30-min interval due to different race time of each subject. Approximately 4 ml of saliva was collected from every subject each time. The samples were collected at least 5 min after intake of any food or fluid to prevent possible contamination and dilution. The samples were stored at 4°C immediately after collection and during shipping to the laboratory for further analysis.

Assay procedures: Saliva testosterone and cortisol levels were measured by radioimmunoassays using commercial kits following the procedures recommended by the manufacturer (Diagnostic Systems Laboratories, Inc. Webster, TX, USA).

Statistical analyses: The differences between pre- and post-race levels were analyzed by paired t-test. The differences between the Young and MA groups were analyzed by t-test. The correlations between variables were analyzed using Pearson's correlation coefficient. All analyses were performed using SPSS for Windows 11.0 (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 is considered statistically significant. All data was expressed as mean±SEM.

Results

The age, height, weight, and race time of the Young and MA groups are shown in Table 1. There was no significant difference in height, weight, or race time between the 2 groups.

The results of pre- and post-race levels and race-induced changes of saliva testosterone, cortisol, and T/C in the Young and MA groups are presented in Table 2. In the Young group, cortisol significantly increased, while testosterone remained unchanged after the race, resulting in significantly lower post-race T/C. In the MA group, testosterone and cortisol levels were both significantly elevated after the race. The degree of increase in cortisol was higher than that in testosterone, resulting in significantly lower T/C after the race. The MA group had significantly lower pre-race testosterone than the Young group. There was no significant difference in the race-induced changes in testosterone, cortisol, or T/C between the 2 groups.

Table 1
Basic characteristics and race time of the young and MA groups (mean+SEM)

Variable	young (n=12)	MA (n=23)
Age (yr)	26.9±1.5	46.3±0.8[a]
Height (m)	1.68±0.08	1.65±0.07
Weight (kg)	60.2±3.5	58.2±3.7
Race time (min)	167.4±6.2	178.3+4.2

[a]p<0.05, Young vs MA.

Table 2
Pre- and post-race levels and race-induced changes of saliva testosterone, cortisol, and T/C in the young and MA groups (mean±SEM)

	Young			MA		
Variable	Pre-race	Post-race	Change (%)	Pre-race	Post-race	Change (%)
Testosterone ($pmol \cdot L^{-1}$)	575.25 ±86.78	624.75 ±95.96	19.90 +14.76	357.35 ±31.62[a]	476.22 ±44.40**	39.17 ±9.94
Cortisol ($nmol \cdot L^{-1}$)	15.15 ±2.90	32.79 ±5.21**	163.87 ±52.11	9.54 ±1.14	27.18 +3.95***	278.68 ±69.25
T/C (x1000)	50.21 ±12.19	24.32 +4.70*	-33.05 ±13.59	49.47 ±6.74	26.68 ±4.48**	-36.95 ±9.16

*p<0.05; **p<0.01; ***p<0.001, post-race vs pre-race; [a]p<0.05, young vs MA.

In the Young group, race-induced change in T/C was significantly correlated with age (r=-0.686, p<0.05) and race-induced change in cortisol (r=-0.729, p<0.01).

In the MA group, race-induced change in T/C was significantly correlated with race-induced change in cortisol ($r=-0.714$, $p<0.01$). Race-induced change in testosterone was significantly correlated with age ($r=0.487$, $p<0.05$) in the MA group. When all subjects were pooled together, race-induced change in cortisol was significantly correlated with race-induced changes in testosterone ($r=0.335$, $p<0.05$) and T/C ($r=-0.517$, $p<0.01$), while pre-race testosterone was significantly correlated with age ($r=-0.386$, $p<0.05$).

Discussion

Salivary concentrations of testosterone and cortisol measured in our study were in the similar magnitude to the previously reported [9,27]. Race-induced change in T/C, an indicator of balance between anabolic and catabolic states, was negatively correlated with age in the Young group. However, this correlation was not present in the MA group. In addition, the race-induced changes in T/C were similar in the 2 groups. It suggested that the decline in T/C after intensive endurance exercise may be more significant with advancing age until mid-30 s, then it may reach the plateau after the fourth decade of life. The recreationally-trained middle-aged subjects in our study were under similar metabolic stress as their younger counterparts after a triathlon.

The MA group had lower pre-race testosterone level than the Young group. Pre-race testosterone level was negatively correlated with age when all subjects were pooled together. These findings were in agreement with others [17,29]. However, the post-race testosterone levels in the 2 groups were similar. It suggested that the age-associated decline in testosterone level disappeared after the race. Furthermore, the race-induced change of saliva testosterone was positively correlated with age in the MA group. Therefore, acute intensive endurance exercise such as triathlon may temporarily normalize the age-related decline in testosterone in recreationally-trained middle-aged men.

Previous researches have been inconsistent with the acute response of testosterone after intensive endurance exercise. It has been shown that testosterone increased after a marathon [9,28], possibly due to the increased gonadotropin-independent testicular production [11] or reduced rate of clearance from plasma [6]. Other investigators have reported no change [20,36] or decrease [13,21] immediately after intensive endurance exercise. The mechanisms of the decreased testosterone levels may include decreased gonadotropin-releasing hormone secretion by hypothalamus [24], enhanced prolactin and inhibited luteinizing hormone (LH) releases by pituitary [15], and/or direct inhibition by cortisol [10]. Our study suggested that the inconsistence may partially result from age differences of the subjects used in these studies. The difference in training status and relative physical stress may be other factors. Schmid *et al.* reported that highly endurance-trained subjects showed an increase, while less-trained subjects showed a decrease in testosterone after a 36-km cross-country skiing [32]. Guglielmini *et al.* showed that serum testosterone remained unchanged after a 20-km competitive walk, increased after a marathon, but decreased after a 107-km ultramarathon [14].

Most studies showed 1.5- to 5-fold of increase in cortisol after intensive endurance exercise, resulting in significantly lower T/C [9,21,28,36]. It appeared that T/C ratio decreased only after relatively intensive endurance exercise, as 2 hours of rowing at approximately 75% of anaerobic threshold did not result in significant change in serum T/C ratio [19]. The current study suggested that race-induce change in cortisol played a more significant role than testosterone in determining race-induced change in T/C. However, our results did not support the inhibitory effect of cortisol on testosterone biosynthesis during a marathon suggested by Cook *et al.* [9], as race-induced changes in cortisol and testosterone were positively correlated. The stimulated synthesis by epinephrine [12] and/or decreased clearance [6] of testosterone during a triathlon may override the inhibition by cortisol in our subjects.

The decreased T/C has been used as an indicator of overtraining [7,31,38] and insufficient recovery [27]. This study suggested that middle-aged subjects were under similar physical stress as their young counterparts. Other researchers have also shown that older and middle-aged trained athletes had similar hormonal responses to younger athletes after 1 hr of exercise at 70% $\dot{V}O_{2max}$ [16] or maximal and submaximal exercise tests [34]

The best effort has been made in this study to ensure that all saliva samples were collected within a short range of time to limit the variation in hormone levels. All subjects awaked at roughly the same time on the race day. The pre- and post-race samples were collected within 20- and 30-min period, respectively.

This study suggested that recreationally-trained middle-aged subjects were under similar imbalance of metabolic states to their younger counterparts after intensive endurance exercise. In addition, the age-associated decline in testosterone could be temporarily normalized by this type of physical exercise in recreationally-trained middle-aged men. Further researches on the physiological implications of the decline in T/C in this group of subjects are needed.

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