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Effect of testosterone in saliva among athletes

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Abstract
The present study was conducted to find out the effect of testosterone in saliva among the national level athletes. In order to reach the result of the study the saliva samples were collected simultaneously for a few days from the national level athletes (N=12), who hailed from the Sports Authority of India, Thalassery. Testosterone is the principal androgenic steroid produced by the testes. Whether of endogenous or exogenous origin, in males and in females, excess testosterone creates an advantage in sports (ACSM, 2006). Studies have shown that testosterone stimulates muscle mass (Storer *et al.*, 2003) and reduces body fat (Bhasin *et al.*, 2004). Athletes aim to maximize their performance through the anabolic effects of testosterone. Due to the dynamic regulation of endogenous testosterone production, including the acute effects of competition and exercise, testosterone concentrations may vary considerably within and among individuals. Generally, cardiovascular exercise and resistance training transiently increase testosterone concentrations in men (reviewed in Hackney [2001], Kraemer and Ratamess [2005]). Testosterone concentrations also vary both before and after competition in a systematic and consistent manner.
The participants were recommended to keep to their normal routine diet throughout the study to avoid dietary effects on the measured variables. The testosterone in the saliva of the participants was measured certain times. In the first day data was collected in the evening. Thereafter, twelve athletes who were selected for the study were asked to do warming up by themselves and then to run for 100 meters dash. The timing of the race by each athlete was recorded. Then the participants of the test were divided into three groups of four members. The first sample of saliva from all subjects was collected before the warming up of the participants in the second day. Then the first group was given a warm up for 15 minutes, the second group 30 minutes and the third group 45 minutes. Then their saliva was collected. Each group was asked to run for 100 meters and there after again their saliva was collected and continued for a few days. Samples were separated using centrifuge, numbered, and then stored at −80°C until used for analysis. When the whole samples were collected, they were brought to a medical lab to test the testosterone level. After getting the results of each saliva samples, they were compared with each other. The data were analyzed by using the statistical method of analysis of variance (ANOVA) and analysis of co-variance (ANCOVA).Whenever the 'F' ratio for the adjusted post test mean showed significance, the Scheffe's test was applied as a post-hoc test to determine the paired mean differences. The level of significance was fixed at 0.05 levels. The result of the study reveals that there is a significant variation in the rising level of testosterone in athletes who have done 30 minutes duration of warming up before hundred meters dash than the other groups.

Keywords: saliva, athletes, warming up saliva, athletes, 100 meters dash & competition

Introduction
Man is inclined to affirm his personality by trying to be distinguished and gain influence and power in his career, in sports and in everyday life, by competing with others. Physical and mental competitive tasks have been found to be associated with higher baseline values and more often with fluctuations of testosterone. Testosterone is the principal androgenic steroid produced by the testes. Whether of endogenous or exogenous origin in males and in females, excess testosterone creates an advantage in sports (ACSM, 2006). Studies have shown that testosterone stimulates muscle mass (Storer *et al.*, 2003) [30] and reduces body fat (Bhasin *et al.*, 2004) [6]. Athletes aim to maximize their performance through the anabolic effects of testosterone. Due to the dynamic regulation of endogenous testosterone production, including the acute effects of competition and exercise, testosterone concentrations may vary considerably within and among individuals. Generally, cardiovascular exercise and resistance training transiently increase testosterone concentrations in men (reviewed in Hackney [2001], Kraemer and Ratamess [2005]) [17, 20, 18]. Testosterone concentrations also vary both before and after competition in a systematic and consistent manner. Recent research has shown a positive relationship of testosterone with aggressive phases of the sports. In sports it has also been reported that winners have higher testosterone levels. (Booth A *et al..*, 1989) [8]. Further studies reveal that baseline testosterone concentrations are positively correlated with the intensity of the aggressive reaction and an increase in testosterone persuades willingness to choose competitive tasks. (Carre JM *et al..*, 2011; Carre JM *et al..*, 2008; Carre JM *et al.*, 2009) [9, 10, 11, 12].
Physical training is a powerful ingredient for immediate

increase in the testosterone concentrations in saliva. Moreover, strength training is an important instrument which stimulates greater levels of testosterone in saliva compared to aerobic training. Testosterone is a powerful stimulator of protein synthesis, specifically in the context of muscle metabolism. Its effects are exerted through the interaction between the hormone and its specific receptor located on the muscle cell. (S. Bhasin, L. Woodhouse and T. W. Storer. 2001) [5]. During the last two decades, the study of testosterone's action on the brain has been revolutionized through the use of functional magnetic resonance imaging (FMRI) and positron emission tomography (PET) which has opened new horizons in the clinical investigation of brain function. With these technologies that permit the visualization and mapping of brain areas when it has been aroused metabolically in response to stimuli, hopes are born of penetrating previously unknown domains of psychic functions and emotions, aspirations similar to those expressed for the exploration of the universe after man first stepped on the moon.

The main mechanism through which testosterone induces protein synthesis is the activation and induction of the proliferation of satellite cells, which subsequently incorporate into muscle fibres, resulting in an increase in the nuclear number. Moreover, this hormone is capable of influencing strength production by stimulating the transition of type II fibres to a more glycolitic profile, increasing the secretion of insulin-like growth factor I, mediated by its influence on the amplitude of growth hormone pulses as well as its influence on the production of neurotransmitters that are important for muscle contraction. (Kaye. K. Brownlee, Alex. W. Moore and Anthony. C. Hackney, 2005) [22]. Testosterone plays a significant role in the arousal of behavioural manifestations in the brain centres involved in aggression, sports performance and on the development of the muscular system that effects their realization. Several field studies have also shown that testosterone increases during the aggressive phases of sports games. In more sensitive laboratory paradigms it was observed that a participant's testosterone rises in the winners of competitions and dominance trials, or in confrontations with factitious opponents. This created the theory that fluctuations of testosterone may be more significant than basal values in the importance of testosterone estimation in relation to aggression. Furthermore, testosterone may promote athletic performance, not only through its long-term anabolic actions, but also through rapid effects on behaviour. Wingfield and colleagues (1990) proposed the "challenge hypothesis", which posits that during mating seasons and times of resource scarcity testosterone concentrations rise to facilitate competition, particularly amongst males. The challenge hypothesis is relevant to human competition in the world of sports (Archer, 2006) [4]. As predicted by the challenge hypothesis, pre-competition concentrations of testosterone rise in male and female athletes in anticipation of the impending competition (Bateup *et al.*, 2002; Booth *et al*., 1989; Edwards and Kurlander, 2010; Mazur *et al*., 1997; Oliveira *et al*., 2009; Salvador *et al.*, 2003; Suay *et al.*, 1999) [8, 19]. In men, testosterone commonly increases following victory and decreases following loss (Booth *et al*., 1989; Elias, 1981; Gladue *et al*., 1989; Mazur *et al*., 1992; Mazur & Lamb, 1980; McCaul *et al*., 1992; Pound *et al*., 2009) [8, 16, 24, 26]. An elite athlete in an international competition is likely to be more engaged and to value victory.

It is possible that high-testosterone individuals have increased motivation to compete in sports. High-testosterone individuals may select into sports as a function of testosterone's positive influence on dominance striving, also known as power motivation (Stanton and Schultheiss, 2009) [29]. Basal testosterone is positively correlated with power motivation in men (Schultheiss *et al.*, 2003; Schultheiss *et al.*, 2005). The positive association between testosterone and power motivation suggests that high testosterone individuals may be the individuals most motivated to pursue athletic competition (e.g., Dabbs *et al.*, 1990a) [14]. In addition to motivating dominance striving, testosterone is positively associated with a number of traits and behaviours that we speculate might foster advantage in competitive sports. Testosterone is associated with enhanced visuospatial ability (Aleman *et al*., 2004) [1] which may provide greater abilities in perceiving critical targets and navigating the physical sports environment, i.e., field, rink, or court. Thus, high endogenous concentrations of testosterone may confer both psychological and physiological advantage in sports. "It also plays a role in body fat, and in muscle size, strength, and function. The investigator took salivary testosterone from twelve national level athletes, who hailed from the Sports Authority of India, Thalassery. The testosterone in saliva was measured four times and brought to a medical lab to test the testosterone level. After getting the results of each saliva samples, they were compared with each other. The data were analyzed in order to find out the impact of warming up for athletic performance in sports. It will give a clue to athletes and coaches about which level warming up is best suitable excellent athletic performance.

Materials and Methods

In order to collect data for the present study, the investigator administrated certain procedures. The data collected continuously for certain days from the saliva samples of 12 national level athletes (N=12) hailed from the Sports Authority of India, Thalassery. The participants were recommended to keep to their normal routine diet throughout the study to avoid dietary effects on the measured variables. The saliva was collected quite a few times. Samples of saliva were taken from the participants during a rest day in the mid-morning, following overnight fasting. The samples were separated using centrifuge, numbered, and then stored at −80°C until used for analysis. Each exercise session consisted of three phases; the warm-up, active, and cool-down phases. During the warm-up phase, the subjects performed simple stretching exercise for all large muscle groups. During the active phase, the subjects were encouraged to run for 100 meters. The last phase was cool-down which continued for 10 to 15 minutes during which the workload gradually decreased until HR and blood pressure had nearly returned to their resting levels. Throughout the entire training session, the subjects were monitored by a portable heart rate monitor to keep the exercise intensity within the precalculated training heart rate for each subject. The time taken for the race by each athlete was also recorded. The participants of the test were

divided into three groups of four members. Then the first group was given a warm up for 15 minutes, the second group 30 minutes and the third group 45 minutes. Then their saliva was collected. Each group was asked to run for 100 meters and again their saliva was collected and this was continued for few days. After collecting the sample, the samples were separated using centrifuge, numbered, and then stored at −80°C until used for analysis. Then they were brought to a medical lab to measure the level of testosterone. Architect i2000 is a fully automatic machine which measures level of testosterone in saliva. The amount of cortisol is shown in nanograms. The serum method was used for sample analyzing processes. After getting the results of each saliva samples, they were compared with each other. The data were analyzed by using the statistical method of analysis of variance (ANOVA) and analysis of co-variance (ANCOVA).Whenever the 'F' ratio for the adjusted post-test mean showed significance, the Scheffe's test was applied as a post-hoc test to determine the paired mean differences. The level of significance was fixed at 0.05 levels. Anthropometric characteristics of the participants (mean ± SD).

Table 1: The analysis of variance for experimental groups on testosterone before the warming up

Groups		Experimental Group 1	Experimental Group 2	Experimental Group 3	SOV	SS	df	MS	'F' Ratio
Before Warming up	Mean	2.95	3.70	4.02	B	2.43	2	1.22	0.93
	SD	1.19	1.17	1.05	W	11.79	9	1.31	

(The table value required for significance at 0.05 level of confidence with df 2 and 9was 3.48)

Table-I shows the mean values of testosterone for three different experimental groups before the warming up as 2.95, 3.70 and 4.02. The obtained '*F*' ratio of 0.93 is lesser than the table value of 3.48. Hence there is no significant difference between the groups. Therefore, there is no significant difference between the groups on testosterone level in saliva before the warming up national level athletes. The mean values of testosterone before the warming up for the three different experimental groups are graphically represented in figure 1.

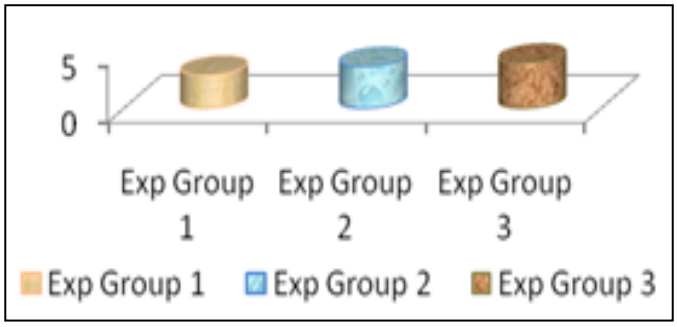

Fig 1

Table 2: The analysis of variance for experimental groups on testosterone after the warming up

Groups		Experimental Group 1	Experimental Group 2	Experimental Group 3	SOV	SS	df	MS	'F' Ratio
After Warming up	Mean	4.27	6.53	8.18	B	30.66	2	15.33	8.64*
	SD	1.51	1.17	1.28	W	15.97	9	1.77	

(The table value required for significance at 0.05 level of confidence with df 2 and 9 was 3.48)

Table-II shows the mean values of testosterone of the three different experimental groups after the warming up as 4.27, 6.53 and 8.18. The obtained '*F*' ratio of 8.64 is greater than the table value of 3.48. Hence there is a significant difference between the groups regarding testosterone levels in saliva. The result of the study reveals that there is significant difference in level of testosterone between the groups after the different duration of warming up. The mean values of testosterone after the warming up of three different experimental groups are graphically represented in figure 2.

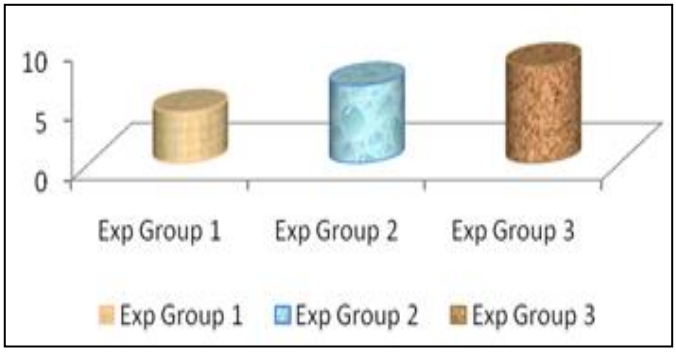

Fig 2

Table 3: The Analysis of Variance for Experimental Groups on Testosterone after the100m Race

Groups		Experimental Group 1	Experimental Group 2	Experimental Group 3	SOV	SS	Df	MS	'F' Ratio
After 100m Race	Mean	6.16	8.60	6.05	B	16.59	2	8.29	5.06*
	SD	1.38	1.11	1.32	W	14.75	9	1.64	

(The table value required for significance at 0.05 level of confidence with df 2 and 9 was .48)

Table-III shows the mean values of testosterone of three different experimental groups after the warming up as 6.16, 8.60 and 6.05. The obtained '*F*' ratio of 5.06 is greater than the table value of 3.48. Hence there is a significant difference between the groups. The result reveals that, there is significant difference in the level of testosterone between the groups after

the 100m dash. The mean values of testosterone after the 100m race for the three different experimental groups are graphically represented in figure 3:

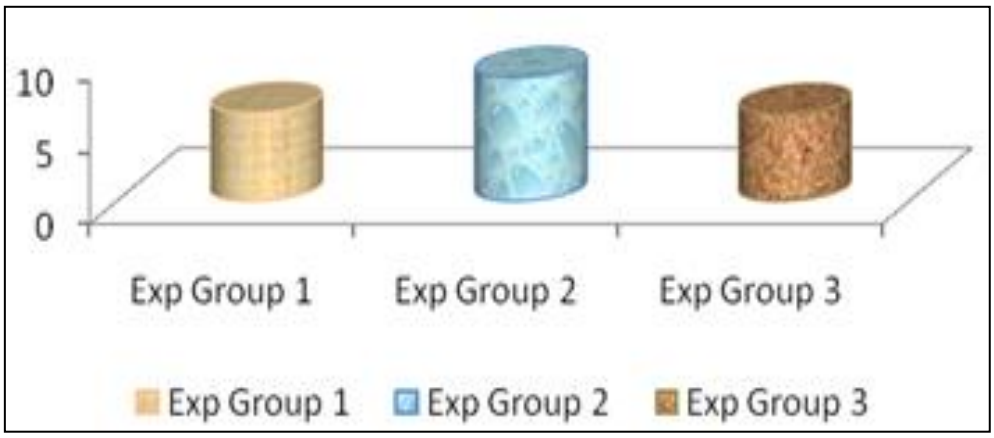

Fig 4

Table 4: The Analysis of Covariance for Experimental Groups on Performance of 100 M Race

Groups		Experimental Group 1	Experimental Group 2	Experimental Group 3	SOV	SS	df	MS	'F' Ratio
Pre Test	Mean	11.16	11.17	11.12	B	0.005	2	0.003	0.48
	SD	0.054	0.066	0.06	W	0.051	9	0.006	
Post Test	Mean	11.16	11.05	11.29	B	0.108	2	0.054	5.87*
	SD	0.068	0.031	0.15	W	0.083	9	0.009	
Adjusted Post Test	Mean	11.15	11.04	11.30	B	0.013	2	0.062	7.55*
					W	0.066	8	0.008	

(The table value required for significance at 0.05 level of confidence with df 2 and 9 was 3.48 and df2 and 8 was 3.68The table-IV shows the mean values of 100m race of three different experimental groups before different duration of warming up as 11.16, 11.17 and 11.12. The obtained '*F*' ratio is lesser than the table value of 3.48. Since after the different duration of warming up and the adjusted post-test '*F*' value of 5.87 and 7.55 is greater than the table values of 3.48 and 3.68. Hence there is a significant variation in the level of performance before and after the specified durations of warming up. The results of the study reveal that the different duration warming up protocol is positively influencing the quantity of performance between the three different training groups. Further, to find out the paired mean difference Scheffe's test was applied as a post hoc test

Table 5: The scheffe's test for paired mean difference on performance of 100m race

Adjusted Post Test Mean on performance of 100m race			Mean Difference	Confidence Interval (CI)
Experimental Group 1	Experimental Group 2	Experimental Group 3		
11.15	11.04		0.11*	0.04
11.15		11.30	0.15*	
	11.04	11.30	0.26*	

(The table value required for significance at 0.05 level of confidence with df 2 and 9 was 3.48 and df 2 and 8 was 3.68) It was clear from the table-V that, the mean differences of experimental group 1 and experimental group 2, experimental groups 1 and experimental group 3, experimental group 2 and experimental group 3 were 0.11, 0.15 and 0.26. All these values were higher than the CI of 0.04. The effect of these three different duration warm up program significantly varies from each other. As per Table-IV the post-test performance mean of 100m race of experimental group 2 shows far better result than the other two groups. So it can be concluded that, 30 minutes duration warming up is the best method to improve the 100m performance than the 20 minutes and 45 minutes duration of warming up programme.

The quantity of testosterone for three different warming up groups and the quality of performance of 100m also differ from each other. Hence, the findings of the study reveal that longer duration of the warming up leads to reduce the positive support of testosterone for better performance. The mean values of performance of 100m before stipulated time duration warming and after the different duration of warming up for three different training groups are graphically represented in figure 4:

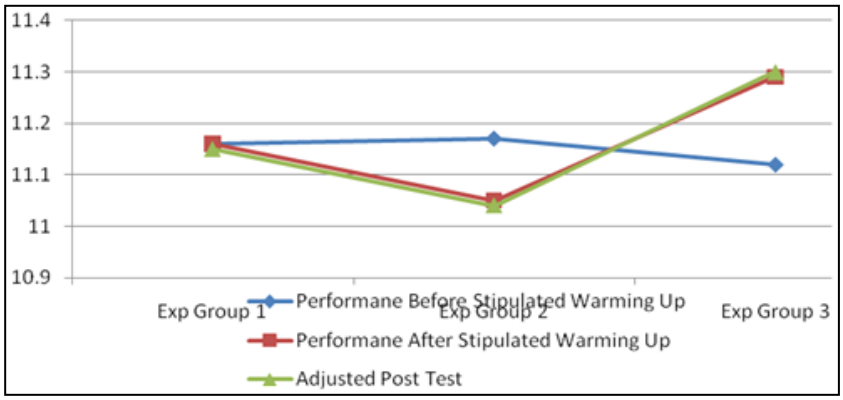

Fig 4: Shows the mean values of 100 m performances before stipulated duration warming up and after the different duration of warming up

Conclusion

The aim of the present study was to analyze difference in the testosterone level in athletes before and after warming up and also to find out which duration of warming up is best suitable for excellent sports performance of athlete. The finding agrees with the findings of the study conducted by V Kathleen, Casto. Christopher, M. Elliott, & David, A. Edwards, (2014) Effects of Warm-up and Racing on Salivary Levels of Cortisol and Testosterone. The finding also conforms to the findings of the study conducted by T. Blair, Crewther, P. Liam, Kilduff, Charlie. Finn, Phil. Scott. Christian, J. Cook, (2016) who examine the salivary testosterone responses to a physical and psychological stimulus and subsequent effects on physical performance in healthy adults. Further, the study also reveals that there is significant difference in the level of testosterone between the groups after the different duration of warming up. 30 minutes warming up produces changes in the concentration of several biologically active molecules including cortisol and testosterone, which play pivotal roles as catabolic and anabolic agents in gluconeogenesis via the proteolytic pathway. The storage of glycogen and muscular protein synthesis is stimulated by more testosterone than15 minutes and 45 minutes warming up program. In short, the result demonstrates that, 30 minutes duration warming up program is the best method to improve 100m performance than the 15 minutes and 45 minutes duration of warming up programme. Moderate 30 minutes warming up for everyday produced significant positive increases in the concentrations of cortisol and free testosterone. Salivary testosterone increases found in this study, coupled with the observed change in the resting cortisol level suggest an enhanced anabolic environment. These results were in agreement with previous studies that reported that aerobic training increased the levels of cortisol and serum testosterone. The increase noted in testosterone was probably induced by adrenaline stimulation, the stimulatory effect of lactate, or the compatibility of testosterone secretion.

Several limitations must be considered when interpreting the results of this study. The major limitation of this study is its low sample size, as only the samples of a few participants could be measured. Also, this study included only males which mean the results are gender specific. Furthermore, the study duration was short, and the study should be repeated for longer periods to measure the effect of long term moderate 30 minutes warming up & its significant increase of testosterone level. The result of the study reveals that there is significant difference in the testosterone level of athletes in different duration of warming up and significant increase in the level of testosterone in athletes who prefer 30 minutets duration of warming up.

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