The Effect of Flat Pyramid Loading Pattern (FPLP) Loading Pattern Weight Training on Salivary Steroids in Male Elite Weight Lifters

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Abstract
Objectives: This study was conducted in order to analyse the effect of flat pyramid loading pattern (FPLP) weight training on salivary steroids levels in strength training athletes. Methods: Eleven elite male weightlifters trained six days per week for one month. Five millilitres of unstipulated whole saliva was obtained pre- and five minutes post-exercise at days first, 15th, and 30th. Results: Resting and post-exercise concentrations of testosterone and cortisol, and the free testosterone to cortisol ratio (FTCR) significantly changed during the training month with higher concentrations of testosterone and cortisol being observed at day 30th. Discussion: It would appear that testosterone and cortisol respond positively and similarly to increasing resistance training load. Conclusions: Our study medicated that the responses of these hormones to exercise depend on volume and intensity of exercise.

Key words: strength training, Testosterone, Cortisol, non-invasive

Introduction
Performance enhancement is the primary focus of exercise training in elite athletes (Maso et al., 2004) and the flat pyramid loading pattern (FPLP) is one the best option if the intent of the training is to develop maximum strength (Bompa and Carrera, 2005). Under-training delays the rate of level of improvement in performance, but overtraining may result negative training adaptations and decreased performance capacity. Thus, determination of optimal intensity and duration of training is vital for athletes and coaches to construct the best training programs. Many investigators have attempted to identify biomarkers of training stress. They have proposed hematological (Varlet-Marie et al., 2003), immunological (Urhausen and Kindermann., 2002), metabolic (Hartmann and Mester, 2000), enzymatic (Brancaccio et al., 2008) and hormonal (Purge et al., 2006) parameters as a diagnostic tools for monitoring training-induced stress. Amongst these biomarkers, the androgenic hormones, such as testosterone have very important roles, because they help regulate gene expression in anabolic and anti-
catabolic processes (Guyton and Hall, 1996). On the other hand, cortisol as a catabolic hormone is a good marker of physiological stress. A number of investigators have subsequently focused on the total and free testosterone, cortisol (Bouget et al., 2006) and free testosterone to cortisol ratio in blood (Adlercreutz et al., 1986; Maestu et al., 2005; Coutts et al., 2007) as valid and reliable hormonal markers of training stress. A high correlation exists between testosterone and cortisol in saliva and blood (serum or plasma) in different situations (Riad-Fafmy et al., 1993; Kumar et al., 2005; Ahn et al., 2007), including prolonged exercise (Lac and Berthone, 2000, McGuigan et al., 2004; Passeleurque et al., 1995). Measurement of salivary testosterone and cortisol concentrations may thus provide a simple non-invasive indicator of current training status. The balance between anabolic and catabolic activity is indicated by the free testosterone to cortisol ratio (FTCR) (Adlercreutz et al., 1986). It is postulated that this ratio can be a useful parameter in the early detection of a misbalance between anabolic and catabolic metabolism (Vervoorn et al., 1992). Whereas the responses of testosterone and cortisol depend on the intensity, duration and type of exercise, the changes of these hormones following an intense exercise bout are controversial. To date, there is little published research, which has measured the incremental salivary testosterone and cortisol in relation with intensity and duration in elite male weightlifters comparing before and after a bout of training, and following a block of training (training program). Thus, the purpose of this study was to examine the FPLP of strength training affect resting (Chronic) and exercise-induced (Acute) salivary testosterone cortisol and FTCR in elite male weightlifters following four weeks of progressive resistive training.

Material and methods

Participants

Eleven elite male weightlifters of the Iranian national team agreed to participate in this study. Descriptive statistics of the subjects’ anthropometric and physiological characteristics are presented in Table 1. All participants were well trained (6 d.wk-1), competing for at least 6 years, and they were regularly participating in international competitions. They participated in the study during the summer training season, which starts after one month of rest from the end of competition season. Before providing a letter of consent to participate in the study, all the subjects were given full information about the study and its purpose. Based on medical examination, all subjects were free from serious disease and did not take any drugs or medication during the study period. Nine participants completed the study.

Table 1. Descriptive characteristics of experimental subject

| Age (yrs.) | 25.6 ± 3.4* |
| Height (cm) | 177± 10.6 |
| Weight (kg) | 87.9 ± 20.3 |
| Body fat (%) | 9.2 ± 4.25 |
| Lean body mass (kg) | 79.57±15.3 |

*Results are expressed as mean ± standard deviation
Training protocol
Participants completed 30 days of training during first period of training camp in preparation for the Asian Games. According to their personal program, subjects trained six days per week. The training program in this period was general, and included snatch, clean and jerk, and power lift, pulling exercises, and squatting. The intensity and duration of training were increased every two weeks for the base of individual programmed. This type of loading pattern (FPLP) starts with a 60 percent of one repetition maximum lift followed by an intermediary set at 80 percent then stabilizes the load at 90 percent for the entire workout. The frequency of training increased from one session per day in the first 2 weeks and two training sessions per day in the next 2 weeks. A standard warm-up and flexibility training program were employed to minimize the probably of injury. All training sessions were conducted indoors to eliminate any effects of changing temperature and humidity. Mean temperature and humidity were 22°C and 45%, respectively (Fig 1).

Fig.1. The experimental design of the study. Arrows (↓) indicated where saliva samples were drawn. † indicated measurement of descriptive characteristics.

Saliva sampling
Before saliva sampling, the participants rinsed out their mouths to remove any substances that can potentially affect salivary testosterone and cortisol measurements. The participants were then asked to drink 500 ml water to avoid dehydration; after which five ml un-stimulated whole saliva was obtained. Participants were instructed to allow saliva to drool into the collecting tubes unaided by spitting. All saliva collections were collected when participants were seated leaned forward, and with their heads down. This procedure was performed pre and 5-min post exercise in days 1, 15th, and 30th of the training block. The day before saliva collecting, each subject was refrained from intensive training and on the experimental day (1, 15, 30) they consumed a standard light breakfast. The entire time line and measurement sequence is graphically illustrated in Figure 1. To reduce the effect of diurnal variations on hormone concentrations, saliva samples were obtained from individuals at the same
time of the day; between 8 a.m. and 10 a.m. Once collected samples were transported in ice to the laboratory for storage prior to analysis. All samples for the hormone determinations were kept frozen at -20°C until use. On the day of testing, all samples were centrifuged at 3000 rpm for 10 min to remove mucus. The clear sample was transported into appropriate testing wells or tubes.

**Hormonal assay**
Enzyme Linked Immunosorbannt Assay (ELISA) using kits obtained from DRG Diagnostics (Germany) measured salivary testosterone and cortisol concentrations. All assays were carried out in duplicate, and the mean value recorded. Quality controls were included in all series of determination. All hormone samples were tested in the same series to avoid any variations among assays.

**Statistical analyses**
Descriptive statistics (means and standard deviations) were determined for all subject characteristics, including age, height, weight, body fat and lean body mass as well as for hormone concentrations. For checking the normality of data, Kolmogorov-Smirnov test was used. The acute effects of training (comparison between pre and post values) on salivary hormones across three measurements phases were analyzed with a paired sample t-test. Chronic effects were analyzed by using one-way analyses of variance for repeated measures. In case of having significant changes we used Bonferoni correction. Epsilon greenhouse-gazer was used if the Mauchly's Test of Sphericity is significant. Effect size was calculated to indicate the magnitude of each treatment (training-program) effect except when otherwise indicated, an alpha level of 0.05 was accepted as indicating significance. All statistical analyses were performed by using SPSS 18 (PASW Statistics 18) computer program.

**Results**

**Results of testing**
Serial resting and post exercise hormonal concentrations. Figure 2. (Panel A) depicts the changes in serial resting and post exercise concentrations of salivary testosterone at 1, 15th and 30th days during training. For resting testosterone concentrations ANOVA indicated a significant change at 30 days (F2, 20 = 58.55, p<0.001, ε=0.583, μ2=0.854).

Paired t test showed that testosterone concentrations were significantly increased on day 30th compared with first day (t=6.59) and day 15th (t=8.74).

For post exercise testosterone concentrations ANOVA indicated a significant change during the training period (F2, 20=71.59, p<0.001, ε=0.586, μ2=0.877). Paired t testing revealed that post exercise testosterone concentrations significantly increased in 30th day in comparison with the first day and 15th day (t=7.58, p<0.001) and 15th day (t=9.26, p<0.001).

For cortisol, (Figure 2, Panel B) depicts the changes in serial resting and post exercise concentrations of salivary cortisol at first, 15th and 30th days during training. For resting cortisol concentrations ANOVA indicated a significant change during training (F2, 20=23.47, p<0.001, ε=0.758, μ2=0.701).

Paired t testing showed that cortisol concentrations were significantly increased on 30th day compared to the first day (t=2.67, p<0.023) and 15th day (t=6.27, p<0.001). The pattern of post exercise cortisol concentrations changes was similar with resting concentrations but post-exercise values were higher than resting values. For post exercise concentrations, ANOVA indicated a significant change during 4 weeks training (F2, 20=52.64, p<0.001 ε=0.547, μ2=0.840). Paired sample t-test showed that post-exercise cortisol concentrations significantly increased in 30th day in comparison with the first day (t=3.63, p<0.005) and 15th day and 15th day in
comparison with the first day ($t=5.57$, $p<0.001$).
The results of Testosterone to cortisol ratio (Figure.2Panel C) indicated that resting levels of this ratio did not significantly change during four weeks of training ($F_2, 20=2.33$, $p=0.122$, $\epsilon =0.556$, $\mu_2=0.190$). In contrast, the post-exercise of FTCR change during training ($F_2, 20=38.54$, $p<0.001$, $\epsilon =0.830$, $\mu_2=0.923$). Paired sample $t$-tests indicated that by the 15th day this ratio had significantly decreased compared with the first day ($t=3.65$, $P<0.004$). By the 30th day this had increased again in compared with 15th day ($t= 8.18$, $P<0.001$).

**Acute hormonal response**

Pre and post exercise testosterone levels were not significantly different pre- vs. post-exercise in the first day and 15th days. However, by the 30th day it was significantly higher post-exercise than pre-exercise ($t=5.92$, $p<0.001$). Comparing pre and post exercise cortisol levels indicated that a single bout of exercise significantly increased the concentration of this hormone in saliva on each of the first ($t=5.21$, $p<0.001$), 15th ($t=4.59$, $p<0.001$) and 30th ($t=2.84$, $p<0.018$) days. Paired sample $t$-test showed that one bout of exercise in the first day ($t=3.14$, $p<0.01$), and 15th day ($t=2.24$, $p<0.036$) caused significantly decreased in testosterone to cortisol ratio.

**Discussion**

This study was performed to examine the effects of one-month FPLP weight training on salivary steroid concentrations pre and post-exercise. Our data revealed that in elite male weightlifters, levels of testosterone significantly increased during and after 30 days intense training. However, there was a relative increase in pre- versus post-exercise difference only after 30 days of training. That is, the only significant acute exercise-induced increase in testosterone was found after the most intense training session. Thus, these data suggest an increasable effect of daily intense training on salivary testosterone after resistance training; finding which was in accordance with previous studies (Häkkinen and Pakarinen, 1993; Raastad et al., 2000). It is likely that a certain level of technique and strength must be reached in training before an acute bout of training can be hard enough to provide sufficient stimulus to induce significant testosterone release. Although the mechanisms behind the testosterone changes following exercise are not known, metabolic alterations could be responsible for these changes. The dominant energy system in heavy resistance training is anaerobic, resulting in lactate production by the larger motor units and release to bloodstream is increased. Lu et al (1997) showed that elevation in blood lactate concentration caused increase cAMP in Leydig cells and then stimulated testosterone production in vivo both after lactate infusions and after exercise training in rats (Lu et al., 1997). On the other hand decrease pH and increased temperature is common during exercise and can altered binding protein affinity, which could result in overall less carrier protein uptake thereby increasing the free testosterone concentration (Rosner., 1990). Since saliva contains only free testosterone (James and Dabbs, 1991), the increased salivary testosterone seen in the present study could be explained by this mechanism. Yet another explanation for the increased testosterone may be related to adrenergic stimulation of Leydig cells, which may in turn increase testosterone production (Hoffman et al., 1994). Unfortunately circulating catecholamine concentrations were not measured so any explanation for our observations remains purely speculative (Fahrner and Hackney, 1998).

Another important finding of this study was that the resting concentration of cortisol was elevated in 15th and 30th day
compared with the first day and post concentrations changed in the same manner. Different mechanisms could explain the increasing concentration of cortisol following exercise, such as increased rates of secretion and/or decreased cortisol metabolism. Cam and Basset, (1983) suggested that rapid increase in cortisol secretion is due to release of stored cortisol from adrenal cells, whereas significant and observable increase in cortisol concentration is due to delayed production (Cam and Basset, 1983).

Stimulating the hypothalamus-pituitary-adrenal axis (HPA) and increasing ACTH secretion from the pituitary gland are the most important factors in stimulation of cortisol secretion. The intensity of training is one of the important and effective factors on HPA axis. For activating this axis the intensity of training should be above a threshold; one which likely requires significant anaerobic metabolism (Buono et al, 1986; Snegovskaya and Viro, 1993). The production of anaerobic metabolism such as accumulation of lactate, decreasing of PH and hypoxia are the stimuli of HPA axis, which ultimately stimulates ACTH secretion. This would explain our observation that maximal salivary cortisol levels were observed at the 30th day following the most intense exercise session. In present study, although lactate concentration was not measured we are confident it would have been elevated.

**Fig.2.** Concentrations of saliva testosterone (nmol.ml)(A), cortisol(nmol.ml)(B) and FTCR(C) at rest and immediately after exercise during four weeks preparatory period. * Denotes statistical differences from day 1; † Denotes statistical differences from day 15; ‡ Denotes statistical differences between the pre and post value. Values are mean±Std. See text for explanation of significant effect.
during training and so cortisol secretion may have increased due to a pH decrease in the blood. On the other hand, accumulation of lactate and increased body temperature may facilitate hormone separating from its carrier protein (Mcmurray et al., 2004) and as a result, the free hormone concentration is increased. In present study, since free cortisol is present in saliva, the increased salivary concentration we observed may be due to the increased release of cortisol from its carrier protein.

The factors that stimulate the HPA axis also stimulate sympathetic-adrenal-medullary axis (SAM) and cortisol secretion is increased. Cortisol secretion is regulated by hypothalamus centers that receive stimulating signals from central nervous system. These signals are modified in a complex system by serotoninergic, adrenergic and dopaminergic systems and other central factors (Björntorp and Rosmond, 2000). Exercise is a suitable stimulus for ACTH secretion as a central nerves system (CNS) hormone that will ultimately stimulates cortisol secretion.

In the present study both rest and post exercise levels of FTCR at the end of the period of training (the 30th day) were significantly higher than the beginning and the middle period of training. In a mathematical context, this increase may be due to increasing testosterone and/or decreasing cortisol concentrations. From a physiological aspect, increasing of this ratio shows a preference of anabolic versus catabolic processes. Previous studies have suggested that a decrease in this ratio indicates the dominance of catabolic processes, and possibly overtraining. Perhaps changes in this ratio after 30 days of training are due to normal hormonal adaptation to resistance training (Banfi et al., 1993).

**Conclusions**

In present study salivary steroids, show significant responses to changes intensity and volume of resistance training in form of flat pyramid loading pattern, but that acute exercise changes in testosterone require sufficient fitness to enable exercise hard enough to stimulate its release. Salivary testosterone and cortisol measures have adequate sensitivity to changes in training load and thus could be used to indicate the physiologic load. In this regard, it may be possible to use measurements of saliva steroids to ensure an appropriate training load is being prescribed.

**References**


