RELATIONSHIP BETWEEN CIRCULATING CORTISOL AND TESTOSTERONE: INFLUENCE OF PHYSICAL EXERCISE

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ABSTRACT

Human research has shown the administration of cortisol into the circulation at rest will result in reduced blood testosterone levels. Many researchers have used these results to imply that physical exercise induced cortisol increases would perhaps result in subsequent reductions in circulating testosterone levels. Our purpose was to examine this concept and determine what, if any, relationship exists between circulating cortisol (C) and testosterone (T) in men (n = 45, 26.3 ± 3.8 yr) at rest and after exercise. Blood samples were collect at rest (10 hour post-prandial; denoted as 'Resting'; n = 45) and again on the same day at 1.0 hr into recovery from intensive exercise (denoted as 'Exercise Recovery'; n = 45). Approximately 48-96 hr after this initial (Trial # 1) blood collection protocol the subjects replicated the exact procedures again and provided a second Resting and Exercise Recovery set of blood samples (Trial # 2). Blood samples from Trial # 1and Trial # 2 were pooled (Resting, n = 90; Exercise Recovery, n = 90). The blood samples were analyzed by radioimmunoassay for C, total T (TT), and free T (fT). Pearson correlation coefficients for the Resting samples ([TT vs. C] r < +0.01; [fT vs. C] r = +0.06) were not significant (p > 0.05). For the Exercise Recovery samples ([TT vs. C] r = -0.53; [fT vs. C] r = +0.21) correlation coefficients were significant (p < 0.05). The findings indicate that exercise does allow the development of a significant negative relationship between C and TT. Interestingly, a significant positive relationship developed between C and fT following exercise; possibly due to an adrenal cortex contribution of fT or disassociation of fT from sex hormone binding globulin. The detected *in vivo* relationships between C and T, however, were associative and not causal in nature and were small to moderate at best in strength.

KEY WORDS: Glucocorticoids, androgens, exercise.

INTRODUCTION

Cortisol, the main glucocorticoid form in humans, is a catabolic hormone secreted from the adrenal cortex in response to physical and psychological stress. Exercise at 60% or more of an individual's maximal oxygen uptake (VO_{2max}) is one of the physical stressors that can cause an increase in the secretion of cortisol (Bloom et al., 1976; Davies and Few, 1973). While cortisol increases during exercise, most of the changes and perhaps effects of this hormone occur after exercise during the early recovery (Daly et al., 2004; Hackney and Viru, 1999; McMurray and Hackney, 2000). Cortisol's release affects metabolism by attempting to help maintain blood glucose levels during physical exercise; it does this in part by acting upon skeletal muscle and adipose tissue to increase amino acid and lipid mobilization (Galbo, 2001; Wolfe, 2001). Cortisol also aids this process by stimulating the liver to produce enzymes involved in the gluconeogenic and glycogenetic pathways allowing conversion of amino acids and glycerol into glucose and glycogen (Galbo, 2001; Wolfe, 2001).

Testosterone is considered a key anabolic hormone with multiple physiological functions in the human body. In males, testosterone is mainly produced and secreted from the Leydig cells of the testes. With respect to exercise, testosterone is especially important in the growth and maintenance of skeletal muscle, bone, and red blood cells (Zitzmann and Nieschlag, 2001). Somewhat similar to cortisol, testosterone increases linearly in response to exercise once a specific intensity threshold is reached with peak concentrations usually occurring at the end of exercise (Wilkerson et al., 1980). It should be noted; however, even low intensity exercise if prolonged enough in duration can result in significant elevations in testosterone (Galbo et al., 1977; [the same is true for cortisol, Väänänen et al., 2002]).

Previous research has established that under certain circumstances a negative relationship exists between the hormones cortisol and testosterone. Bambino and Hsueh (1981) showed a direct inhibitory effect of high doses of glucocorticoids upon testicular Leydig cell function in rats, which resulted in a decrease in the production of testosterone. Cumming et al. (1983) found a similar relationship in humans, using pharmacological doses of cortisol to induce a decrease in testosterone production. These latter researchers speculated cortisol disrupted the testicular testosterone production process (i.e., via disruption of the hormone's biosynthesis pathway).

While this previous research has identified this inverse, negative relationship between cortisol and testosterone, studies thus far have apparently not statistically examined this point closely or have done so with inadequate sample sizes. Also, while pharmacological doses of cortisol appear to have a strong effect on circulating testosterone concentrations in humans, the strength of the *in vivo* relationship at rest and in recovery from exercise has not been thoroughly determined. This last point is important as many published exercise physiology studies have inferred that observed testosterone reductions following certain forms of physical exercise are caused by cortisol elevations in response to the exercise. Therefore, the purpose of this study was to determine what, if any, relationship exists between physiological levels of cortisol and testosterone (both in total and free forms) in men at rest and in recovery from physical exercise. We approached this research question using a simple correlative design, which would allow us to examine whether a relationship existed between the hormones. This approach, however, only permitted us to determine if the relationship was of an associative nature and not a "cause and effect".

METHODS

A total of 45 healthy, physically active men (4-5 d·wk^{-1}, >60 min·d^{-1}, ≥5 yr) participated in this study. Their physical characteristics were as follows: age, 26.3 ± 3.8 years [mean ± SD]; height, 1.77 ± 0.06 meters; and body mass, 77.2 ± 7.8 kilograms (kg). All subjects gave voluntary, written informed consent prior to participation in the study in accordance with the Helsinki Declaration.

These men were all participants in several resting and exercise based research ancillary-studies which involved giving multiple blood samples. From each subject blood samples were collected at rest in a 10 hour post-prandial state (denoted as 'Resting'; n = 45) and again on the same day at 1.0 hr (0.75 - 1.25 hr, range) into recovery from intensive exercise (denoted as 'Exercise Recovery'; n = 45). The recovery sample time was based upon research by Daly et al. (2004) in which recommendations were made concerning sampling time for obtaining peak cortisol responses. Approximately 48-96 hr after this initial (Trial # 1) blood collection protocol the subjects replicated the preceding procedures providing a second (Trial # 2) Resting and Exercise Recovery set of blood samples. In the time between the collection of the Resting and Exercise Recovery blood specimens in both Trial # 1 and Trial # 2 only water was consumed (*ad lib*). These overall procedures are depicted in Figure 1.

All blood samples were collected by the veni-puncture technique (~3 mL blood), a procedure all the subjects were familiar with from previous research participation. All blood samples were collected in the mid-morning time frame (9:00-11:30 AM), and the Resting and Exercise Recovery blood collections times for Trial # 1 and Trial # 2 were replicated (within ± 0.25 hr, range). The exercise protocols performed by the subjects varied dependent upon the focus of each ancillary study in which they were participants. The modes of exercise involved running, rowing, and cycling, which were all of an intensity (~65% to 75% VO$_{2max}$) and duration (60 - 90 minutes) sufficient to cause a significant increase in cortisol secretion (see Table 1; Davies and Few, 1973; McMurray and Hackney, 2000). Additionally, the mode, intensity and duration of the exercise at Trial # 1 and Trail # 2 were replicated.

Once collected, blood specimens were placed on ice until centrifuged at 4°C (3000 x g - 15 min) to separate the serum. The sera were in turn stored frozen at -80°C until later analysis. Hormonal analysis was conducted by using single-antibody solid phase radioimmunoassay procedures using commercially available I^{125} kits for the
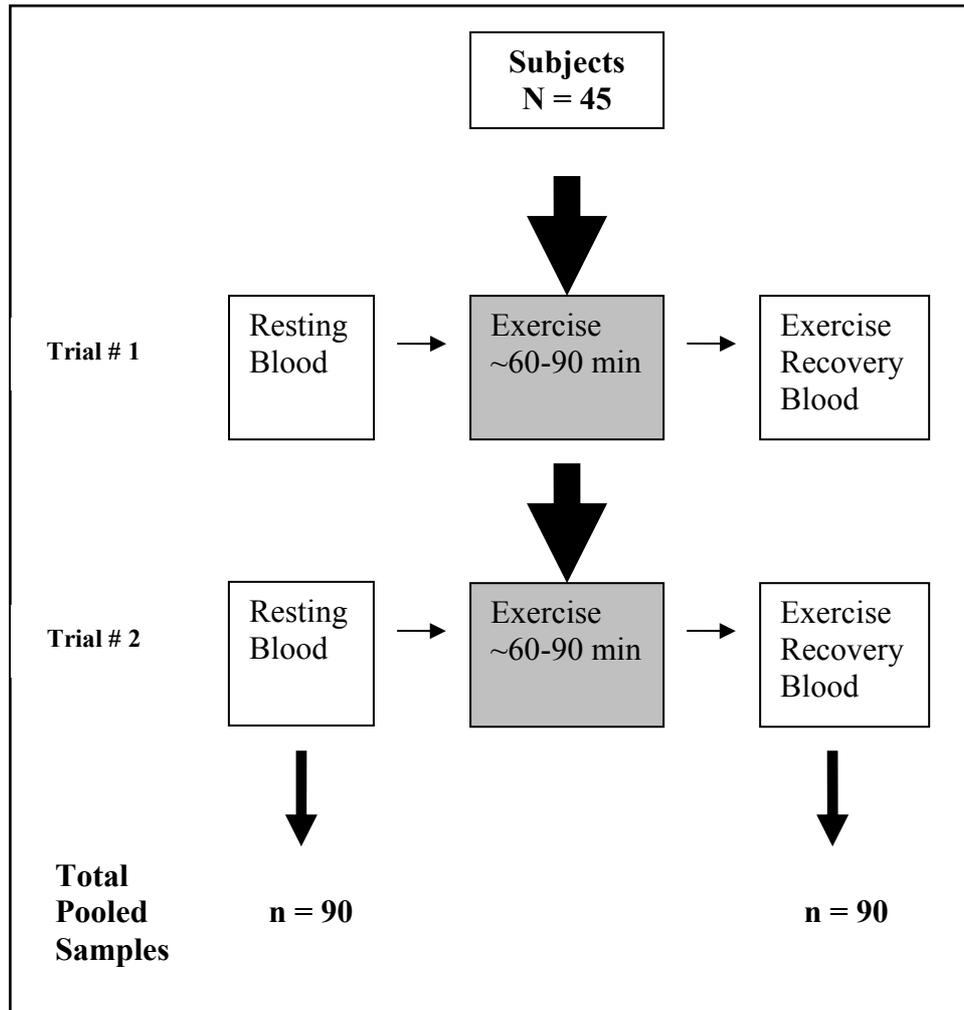
Figure 1. The figure is a schematic representation of the blood sampling protocol used in the study. The blood samples were collected in the mid-morning time frame (9:00-11:30 AM). The Resting and Exercise Recovery blood collections times for Trial # 1 and Trial # 2 were replicated (within ± 0.25 hr).

determination of cortisol, total testosterone, and free testosterone (DPC Inc, Los Angles, CA, USA). The within assay coefficients of variance ranged from 5.9% to 8.1% while the between assay coefficients of variance ranged from 6.5% to 9.8%.

Statistical analyses were performed using the Statistica software (version 6.0) program (Statsoft, Tulsa, OK, USA). Descriptive statistics were determined for all subject characteristics, including age, height, and weight, as well as for hormone concentrations. The hormonal data from Trial # 1and Trial # 2 were pooled so that the Resting and Exercise Recovery comparison involved 90 data points each. Repeated measures ANOVA were applied to the 'Resting' versus the 'Exercise Recovery' to examine for concentration differences within each respective hormone. Relationships between hormone concentrations were analyzed using Pearson correlations, and Hotelling statistics were used to test for significant difference between the correlation coefficients (Cohen, 1988). Statistical significance was set at $p \leq 0.05$.

Table 1. Exercise activities of the subjects* used within the research study.

Exercise Activities	Exercise Duration (min)	Exercise Intensity (%VO$_{2max}$)	Sample Size (n)
Running	60	~70-75%	30
Cycling	90	~65%	12
Rowing	60	~65%	3

*Data sources: Farhner and Hackney, 1998; Hackney et al., 1995; Hackney and Viru, 1999; Matuszkiewicz et al., 2001; previously unpublished data.

RESULTS

Descriptive results of the hormone concentrations for the Resting and Exercise Recovery samples are displayed in Table 2. All hormonal values within each set of samples were normal and within acceptable clinical ranges for the conditions under which they were collected (McMurray and Hackney,

2000; Tietz, 1990). Cortisol and free testosterone concentrations were found to be significantly greater in the Exercise Recovery samples than the Resting samples (p < 0.01) in agreement with previous literature (Bonifazi et al., 1996; Davies and Few, 1973; Kivlighan et al., 2005; Vogel et al., 1985).

Table 2. Concentrations of cortisol, total testosterone (TT) and free testosterone (fT). Data are means (±SE).

	Cortisol (nmol·L^{-1})	TT (nmol·L^{-1})	fT (pmol·L^{-1})
Resting (n=90)	229 (11)	16.3 (.7)	73 (3)
Exercise Recovery (n=90)	596 (22) *	14.6 (1.0)	101 (4) *

* denotes significant (p ≤ 0.01) difference within a specific hormone from the respective Resting value.

Pearson correlations were used to analyze the relationship between cortisol versus total testosterone and free testosterone. Of the four correlations generated, two were significant, although the magnitude of effect size was small to moderate for these coefficients (Cohen, 1988). Cortisol versus total testosterone was negatively related in the Exercise Recovery samples (r = -0.53, p<0.001, see Figure 2). The Resting samples showed no relationship for cortisol versus total testosterone (r < 0.01, p > 0.05). The cortisol versus free testosterone was positively related in the Exercise Recovery samples (r = 0.21, p < 0.05, See Figure 3). The Resting samples showed no relationship for cortisol versus free testosterone (r = 0.06, p > 0.05).

For the cortisol versus total testosterone relationship, the Exercise Recovery coefficient was significantly greater than the Resting coefficient (r = -0.53 vs. r < 0.01, respectively; Hotelling statistic, p < 0.01). Likewise, for the cortisol versus free testosterone the Exercise Recovery coefficient was significantly greater than the Resting coefficient (r = 0.21 vs. r = 0.06, respectively; Hotelling statistic, p < 0.01).

DISCUSSION

The purpose of this study was to determine what, if any, relationship exists between cortisol and testosterone (both in total and free forms) in physically active men at rest and in the recovery
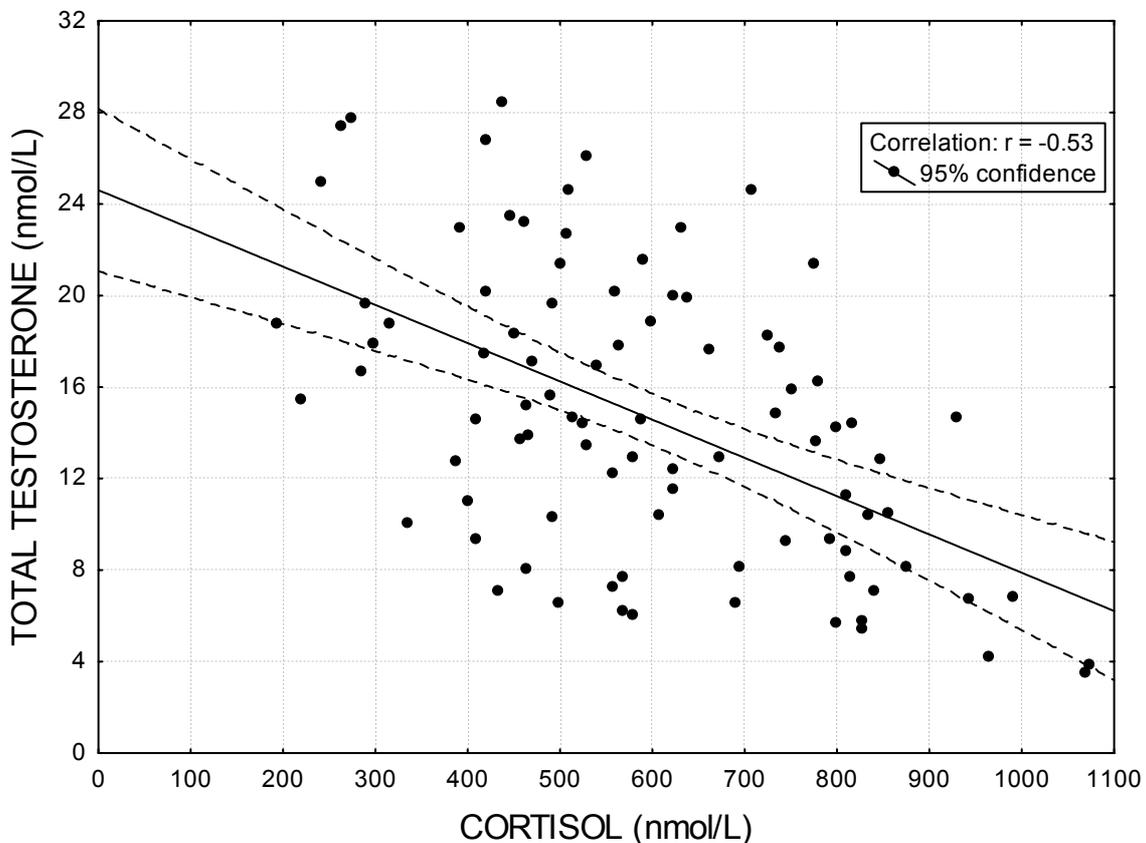

Figure 2. Total testosterone and cortisol in the Exercise Recovery blood samples (n=90). The correlation coefficient is statistically significant (p<0.001).
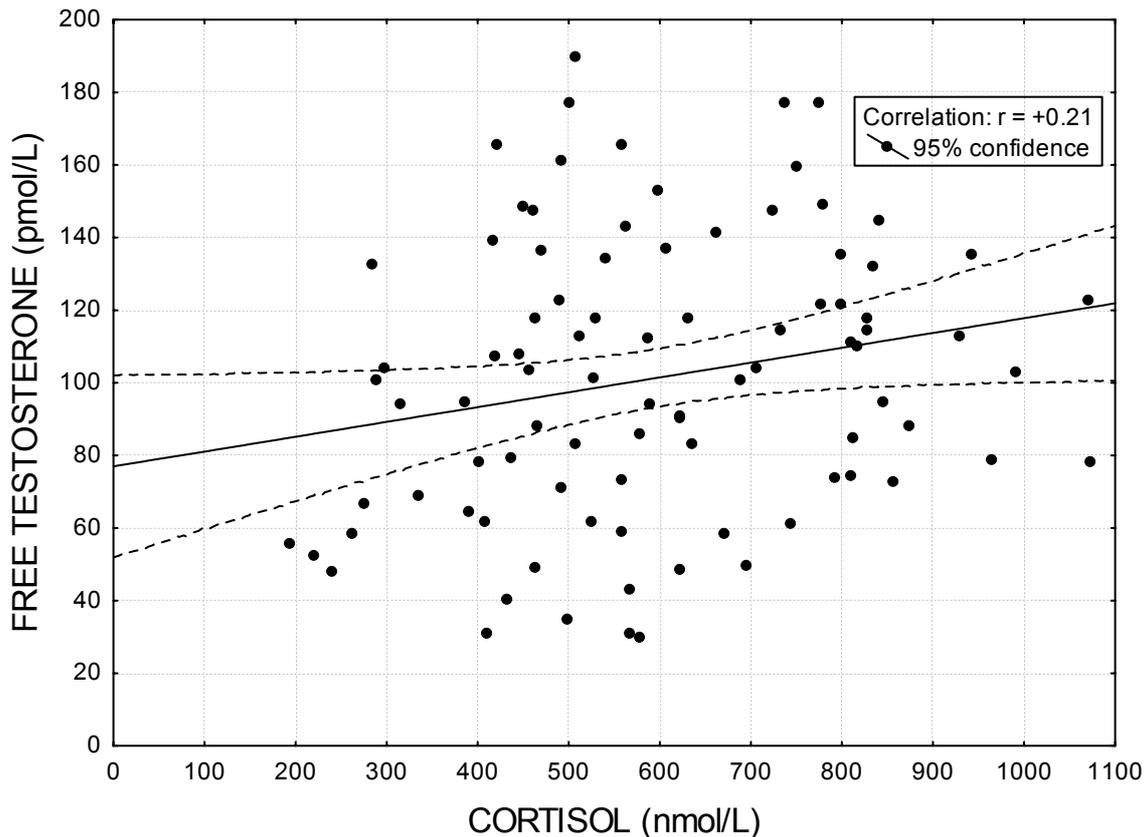
Figure 3. Free testosterone and cortisol in the Exercise Recovery blood samples (n=90). The correlation coefficient is statistically significant (p<0.05).

from physical exercise. Previous research had found that pharmacologically manipulated levels of cortisol had resulted in reductions in circulating testosterone (*i.e*., a negative relationship) (Bambino and Hsueh, 1981; Cumming et al., 1983). We hypothesized that this negative relationship would exist between circulating cortisol and testosterone, specifically after exercise when cortisol was at the high end of the normal physiological range.

A correlation relationship was found in this study that was in concordance with previous literature, and supportive of our hypothesis. That is, a significant negative relationship between cortisol and total testosterone was found in the Exercise Recovery samples; however, there was no relationship between the hormones in the Resting samples. This finding of a relationship when cortisol is elevated (~160% above Resting) suggests that perhaps that some critical level of cortisol must be reached in order to substantially influence circulating testosterone levels (see mechanism discussion below concerning pharmacological dosages). This notion, however, is in need of further examination in future research.

This negative association between testosterone and cortisol has been reported in a few other exercise related studies (Hoogeveen and Zonderland, 1996; Nindl et al., 2001; Opstad, 1992). However, these studies have suffered from use of very small

samples sizes (thus limiting their external validity) or they reported the relationship only on an observational basis and did not test the association statistically. To our knowledge, our study is the first to examine this issue in an exercise-based study with adequate subject numbers to allow a statistical analysis of the relationship. It is recognized, however, that the issue of blood collection timing in the present study is a limitation within our data. Ideally, the blood sampling times should have been precisely identical and standardized in Trial #1 and # 2 as has been recommended by others (Daly et al., 2005). Nevertheless, the Exercise Recovery data for cortisol and total testosterone in the present study reveal similar relationships as reported by the Daly et al. study (2005).

The specific physiological basis for a negative relationship between cortisol and total testosterone can not be determined from our data, but several postulates have been put forth by other investigators. Cumming et al., (1983) examined the response of luteinizing hormone (LH), prolactin, and total testosterone to cortisol administration (*i.e.*, pharmacological levels). They found that while circulating testosterone was decreased, there were no changes in LH or prolactin, suggesting that the effect of cortisol on testosterone production was in the testes and not the central endocrine regulatory components (*i.e.,* hypothalamus-pituitary; [The

authors, however, did not measure LH pulse frequency or amplitude, thus a central component influence can not be totally discounted]). Cumming and associates speculated that cortisol disrupted the testicular steroidogenic process in the Leydig cells perhaps by enzymatic inhibition. *In vitro* animal model research supports this conjecture on their part. For example, Bambino and Hsueh (1981) found a direct inhibitory effect of infused pharmacological dosages of cortisol on the LH receptor activity and content of the testes in rats. Specifically, they proposed that that increased concentrations of glucocorticoids (cortisol) disrupt the binding of LH on the testes and thus steroidogenesis process. Later work by these same authors expanded on this point and suggested that perhaps testicular cAMP production and the activity of the 17α-hydroxylase enzyme were somehow suppressed by glucocorticoids (Welsh et al., 1982). Other researchers have reached similar conclusions; *i.e.*, the steroidogentic enzymatic activity for testosterone synthesis within the testis is disrupted (Castro and Matt, 1997). We acknowledge also that other factors could be affecting cortisol and total testosterone in an independent fashion, and thus the observation we report could be the result of such factors and the cortisol and testosterone in our subjects are not directly affecting one another.

A negative relationship was not found between cortisol and free testosterone, but rather the opposite occurred - a positive relationship existed in the Exercise Recovery samples. This finding has been previously reported in the literature (Daly et al., 2005). The physiological explanation for this finding is uncertain, but several possibilities exist. First, it is possible that the increased concentration of free testosterone is a result of an increased adrenal contribution of testosterone to the circulation. In response to physical stress such as exercise, the adrenal gland is stimulated through a cascade of reactions to produce cortisol. Cortisol and testosterone are formed in the same cascade of reactions at the adrenal gland (Kroboth et al., 1999). Therefore when the adrenal gland is stimulated to produce cortisol, it is possible that some free testosterone is produced and secreted concurrently, leading to increased circulating concentrations of both hormones. Secondly, testosterone can be transported in the blood bound to sex hormone binding globulin and other carrier proteins (e.g., albumin), while cortisol can be transported somewhat by the latter and cortisol binding globulin (Rosner, 1990). Since, cortisol and testosterone are formed from the same precursor, they are structurally very similar. Thus the possibility exists that an increased concentration of cortisol in circulation could cause some dissociation of free

testosterone from its carrier proteins as the two hormones compete for binding sites (Rosner, 1990). Additionally, binding protein affinity changes can happen in response to the pH and temperature changes occurring with exercise which could result in overall less carrier protein uptake of hormone thereby increasing the free hormonal portion (Obminiski and Stupnicki, 1996; Rosner, 1990). Whether any of these events occurred to influence the hormones and resulted in the positive relationship observed is uncertain and speculation on our part; further research is necessary to examine this phenomena and elucidate the mechanism.

CONCLUSIONS

In conclusion, there are statistically significant relationships between cortisol and testosterone in humans in the recovery from physical exercise. Previous results demonstrate pharmacological levels of cortisol have a highly significant negative effect on circulating testosterone concentrations (Bambino and Hsueh, 1981; Cumming et al.,1983). Exercise appears to allow for the development of a similar negative relationship between cortisol and total testosterone (although not an extremely robust association). This finding would seem to support the notion that the observed testosterone reductions following certain forms of physical exercise could be related to cortisol elevations in response to that exercise. But, this is speculative on our part as the current data only indicate the existence of an associative relationship between the hormones and does not indicate a cause and effect relationship. Further research is necessary to fully address this question. Additionally, it is important for researchers to distinguish between the forms of testosterone being examined when addressing the testosterone-cortisol relationship following exercise, as free testosterone has an opposite relationship with cortisol to that of total testosterone.

REFERENCES

Bambino, T.H. and Hsueh, A.J. (1981) Direct inhibitory effect of glucocorticoids upon testicular lutenizing hormone receptor and steroidogenesis in vivo and in vitro. *Endocrinology* **108,** 2142-2148.

Bloom, S.R., Johnson, R.H., Park, D.M., Rennie, M.J. and Sulaiman, W.R. (1976) Differences in the metabolic and hormonal response to exercise between racing cyclists and untrained individuals. *Journal of Physiology* **258,** 1-18.

Bonifazi, M. and Lupo, C. (1996) Differential effects of exercise on sex-hormone-binding globulin and non-sex hormone-binding globulin-bound testosterone. *European Journal of Applied Physiology* **72,** 425-429.

Castro, W. and Matt, K.S. (1997) Neuroendocrine correlates of separation stress in the Siberian dwarf hamster (Phodopus sungorus). *Physiological Behavior* **61,** 477-484.

Cohen, J. (1988) *Statistical power analysis for the behavioral sciences.* 2nd edition. New Jersey: Lawrence Erlbaum Publishing.

Cumming, D.C., Quigley, M.E. and Yen, S.C. (1983) Acute suppression of circulating testosterone levels by cortisol in men. *Journal of Clinical Endocrinology and Metabolism* **57,** 671-673.

Daly, W., Seegers, C., Rubin, D.A., Dobridge, J.D. and Hackney, A.C. (2005) Relationship between stress hormones and testosterone with prolonged endurance exercise. *European Journal of Applied Physiology* **93,** 375-380.

Daly, W., Seegers, C., Timmerman, S. and Hackney, A.C. (2004) Peak cortisol response to exhausting exercise: effect of blood sampling schedule. *Medicina Sportiva* **8,** 1-4.

Davies, C.T.M. and Few, J.D. (1973) Effects of exercise on adrenocortical function. *Journal of Applied Physiology* **35,** 887-891.

Fahrner, C.L. and Hackney, A.C. (1998) Effects of endurance exercise on free testosterone concentration and the binding affinity of sex hormone binding globulin. *International Journal of Sports Medicine* **19,** 12-15.

Hackney, A.C., Premo, M.C. and McMurray, R.G. (1995) Influence of aerobic versus anaerobic exercise on the relationship between reproductive hormones in men. *Journal of Sports Science* **13,** 305-311.

Hackney, A.C. and Viru, A. (1999) Twenty-four-hour cortisol response to multiple daily exercise sessions of moderate and high intensity. *Clinical Physiology* **19,** 178-182.

Hoogeveen, A.R. and Zonderland, M.L. (1996) Relationship between testosterone, cortisol, and performance in professional cyclists. *International Journal of Sports Medicine* **17,** 423-428.

Galbo, H. (2001) Influence of aging and exercise on endocrine function. *International Journal of Sports Nutrition and Exercise Metabolism* **suppl. 11**, S49-57.

Galbo H., Hummer L., Peterson, I.B., Christensen, N.J. and Bie, N. (1977) Thyroid and testicular hormonal responses to graded and prolonged exercise in men. *European Journal of Applied Physiology* **36,** 101-106.

Kivilighan, K.T., Granger, D.A. and Booth, A. (2005) Gender differences in testosterone and cortisol response to competition. *Psychoneuroendocrinology* **30,** 58-71.

Kroboth, P., Salek, F., Pittenger, A., Fabian, T. and Frye, R. (1999) DHEA and DHEA-A: a review. *Journal of Clinical Pharmacology* **39,** 327-348.

Matuszkiewicz, A,, Kaczor, J., Ratkowski, W., Popingis, J. and Hackney, A.C. (2001) Ineffective training, free-radical formation and low protein diet in Polish sportsman. *Medycyna Sportowa* **17,** S21.

McMurray, R.G. and Hackney, A.C. (2000) Endocrine responses to exercise and training. In: *Exercise and Sport Science*. Eds: Garrett, W.E. and Kirkendall, D.T. Philadelphia: Lippincott Williams & Wilkins. 135-156.

Nindl, B.C., Kraemer, W.J., Deaver, D.R., Peters, J.L., Marx, J.O., Heckman, J.T. and Loomis, G.A. (2001) LH secretion and testosterone concentrations are blunted after resistance exercise in men. *Journal of Applied Physiology*, **91,** 1251-1258.

Obminiski, Z. and Stupnicki, R. (1996) Effects of temperature and pH on the magnitude of the free fraction of cortisol in serum. *Experimental and Clinical Endocrinology and Diabetes* **204,** 350-352.

Opstad, P.K. (1992) Androgenic hormones during prolonged physical stress, sleep, and energy deficiency. *Journal of Clinical Endocrinology and Metabolism* **74,** 1176-1183.

Rosner, W. (1990) The function of corticosteroid-binding globulin and sex hormone -binding globulin: recent advances. *Endocrine Reviews* **11,** 80-91.

Tietz NW. (1990) *Clinical Guide to Laboratory Tests*, 2nd edition. WB Saunders Publishing, Philadelphia.

Väänänen, I., Vasankari, T., Mäntysaari, M. and Vihko, V. (2002) Hormonal responses to daily strenuous walking during 4 successive days. *European Journal of Applied Physiology* **88,** 122-127.

Vogel, R.B., Books, C.A., Ketchum, C., Zauner, C.W. and Murry, F.T. (1985) Increase of free and total testosterone during submaximal exercise in normal males. *Medicine and Science in Sports and Exercise* **17,** 119-123.

Welsh, T.H., Bambino, T.H. and Hsueh, A.J. (1982) Mechanism of glucocorticoid-induced suppression of testicular androgen biosynthesis in vitro. *Biology of Reproduction* **27,** 1138-1146.

Wilkerson, J.E., Horvath, S.M. and Gutin, B. (1980) Plasma testosterone during treadmill exercise. *Journal of Applied Physiology* **49,** 249-253.

Wolfe, R.R. (2001) Control of muscle protein breakdown: effects of activity and nutritional states. *International Journal of Sports Nutrition and Exercise Metabolism* **Suppl. 11**, S164-169.

Zitzmann, M. and Nieschlag, E. (2001) Testosterone levels in healthy men in relation to behavioral and physical characteristics: facts and constructs. *European Journal of Endocrinology* **144,** 183-197.

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KEY POINTS

- Pharmacologically increased levels of cortisol have a significant negative effect on circulating testosterone
- After certain types of physical exercise a negative statistical associative relationship exist between cortisol and total testosterone

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