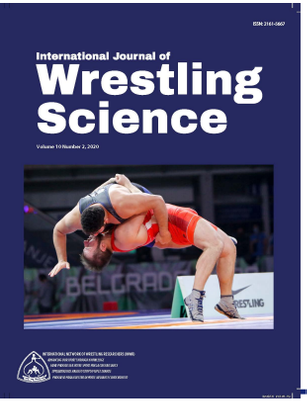

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ENDOCRINE BIOMARKER RESPONSES DURING AN INTERCOLLEGIATE WRESTLING SEASON

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ABSTRACT
The aim of the current investigation was to quantify the endocrine biochemical markers including testosterone (T), cortisol (C), secretory immunoglobin A (sIgA) and testosterone: cortisol ratio (T:C) as indicators of the balance between anabolic and catabolic processes quantifying the homeostatic response to late competitive phase of the college wrestling season using a longitudinal repeated measures design. A volunteer sample of ten collegiate wrestlers (age = 22.11 ± 1.1yrs, height = 177.8 ± 3.1cm; weight = 77.6 ± 20.9kg) gave saliva samples via unstimulated passive drool (@0.75 mL) at 24 hours before and 24-48 hours post competition. All biomarker concentrations were determined using indirect enzyme-linked immunosorbent assay (ELISA) kits. Time since awake for each sample, whether the team traveled, number of competitions, day of the study, and trial (pre vs. post) was recorded to test for effect and interactions on biomarker concentrations. Conceptualizing the change in T, C, T:C ratio and sIgA over the competitive season, a series of hierarchical linear models were utilized. Our analyses revealed that cortisol and testosterone concentrations were predicted by day of study, time since awake, and number of competitions; however, T:C ratio was only predicted by day of the study and trial, indicating that as the season progressed, T:C ratio decreased over time after competitions. Secretory IgA was predicted by number of competitions indicating a decrease in sIgA as bouts increased, sIgA decreased. The exploration of salivary biomarkers could be conceptualized as an objective method to understand demands of competitive exposures on several physiological systems.
Key Words: athletics, combative sports, hormones, physiology, physical fitness, salivary

INTRODUCTION

Wrestling is a vigorous and physically demanding sport activity requiring tremendous physical preparation as well as the ability to tolerate significant psychological and emotional load. Training for wrestling traditionally incorporates high exercise volumes with limited recovery periods in an annual training program. Wrestlers at the elite level are required to sustain physical efforts of intermittent power and endurance at high speed with repeated high intensity bouts in a single match. For the one-day tournament competition, evidence of muscle damage and inflammation response is heightened (Barbas et al., 2011; Kafkas et al., 2016). Over two days of competition, significant reductions in lower and upper body strength and power occurs as the tournament progresses (Kraemer et al., 2001). From these investigations, data suggest neuroendocrine responses are linked to success (Fry et al., 2011). Wrestling has a profound effect on endocrine and cardiovascular function during a competitive season (Schmidt et al., 2005; Strauss et al., 1985). A college wrestling season is a stressor reducing an individual's lean body mass and peak power in competing athletes (Roemmich & Sinning, 1997). High intensity exercise over an extended period of a wrestling season has also been associated with immunosuppression, including a reduction in circulatory lymphocyte concentration and secretory immunoglobin A (sIgA) (Nieman, 2000; Pedersen et al., 1996; Ratamess et al., 2013).

Empirical evidence on physiological and performance changes in competitive intercollegiate athletes during a season, specifically with participants in team sports, is limited (Hoffman et al., 2005). Physical exercise stress as a result of high level training and competition induces significant hormonal, biochemical and immunological changes to an athlete's physiology. Multiple investigations on soccer players, road cyclist and runners demonstrated hormonal changes during a season of competition and major tournaments (Filaire et al., 2001; Hough et al., 2011; Moreira et al., 2009; Ohkuwa et al., 1995). Subsequently, monitoring the immune status of athletes has received greater attention at the elite level with most of the research focusing primarily on post-exercise salivary immune response (Owen et al, 2016).

Testosterone (T), cortisol (C), testosterone-to-cortisol ratio (T:C) and sIgA have been proposed as indicators of the ratio between anabolic and catabolic processes (Viru & Viru, 2004). These biomarkers have been proposed

as indicators of the balance between anabolic and catabolic processes amongst athletes across an annual conference season and tournament campaign (Jack et al., 2013). Testosterone is a male sex hormone that is important for sexual and reproductive development. Cortisol is a hormone that is released from the adrenal gland in response to stress or other chemical signals. Cortisol as a stress marker has been extensively studied in professional, collegiate and recreation athletes due to its catabolic and immunosuppressive effect on protein synthesis (Papa et al., 2015). However, regular exercise-induced training will decrease this effect, causing the body to have a better response to stress and require less C release. Empirical evidence for fatigue-related mechanisms in exercise-induced training is unequivocal, but the function of the hormonal regulation of metabolism and cellular homeostasis is well documented. Repeated exercise-induced training bouts and competition without a sufficient period of recovery can cause a persistent disturbance in this metabolic homeostasis (Morgans et al., 2015). Secretory IgA is an important protein that plays a major role in protecting us from respiratory infections found throughout the body such as the intestines, the lungs and importantly in saliva. Investigations have shown that when the level of sIgA is high, the risk of upper respiratory tract infections (URTIs) is low, and people with low sIgA suffer from URTIs at a higher rate than the general population (Adlercreutz et al., 1986). The causes of immune depression after prolonged exercise are thought to be related to increases in circulating stress hormones (e.g., adrenaline and C).

The primary aim of the current investigation was to describe the changes in salivary biochemical markers including T, C, T:C and sIgA during the latter stages of the competitive season of collegiate-level wrestlers. A secondary aim is to identify the variables that relate to changes in these markers. We hypothesize that due to the demands on wrestlers during this phase of the competitive season, there will be changes in these biochemical markers. Although changes in T and C may reflect the physiological stress associated with weight reduction ("cutting weight") in wrestling, it is expected that changes might appear to amplify the endocrine response to a season of training and competition. This is primarily related to the ability of biomarkers to provide a quantitative view of the homeostatic response in a subject's training as a point of exercise periodization and potential adaptive processes to training (Poste, 2011).

METHODS

Experimental Approach to the Problem

Investigators collected salivary samples simultaneously from a cohort of athletes in a non-laboratory setting, which would cause minimal interference in the athletes' training regimen. In general, the investigators collected samples from each subject approximately 24 hours before competition and 24-48 hours post competition, allowing a competition monitoring program across an entire collegiate conference competitive season. Prior to any data collection, all participants signed an informed consent form. This study was approved by and carried out in accordance to the Declaration of Helsinki.

Participants

To be eligible, each participant was considered elite as a member of a top-10 ranked NCAA Division I wrestling team (5 obtained NCAA All American designation) between the ages of 19 and 24 years of age. Participants were free from any current or ongoing musculoskeletal injuries or neuromuscular disorders via pre-season physical exam by a licensed physician. Subjects who met inclusion criteria were contacted by the investigator. All wrestlers were fully familiarized with the experimental procedures within this investigation. All mandatory health and safety procedures have been complied with in the course of conducting any experimental work. Ten male elite wrestlers (mean ± SD; age =22.11 ± 1.1yrs; height=177.8 ± 3.1cm; weight=77.6 ± 20.9 kg) were analyzed and reported in this manuscript.

Procedures

During the study, subjects were instructed to maintain normal daily food and water intake, with no individual dietary or recovery modality changes made throughout the annual assessment period as determined by a state licensed, registered dietician providing a clearer picture of well-being with respect to immune function, physical state and performance. Wrestlers provided pre-workout saliva sample approximately 15 to 30 minutes before training session commenced. Additionally, before saliva sampling, to regulate saliva secretion, wrestlers were asked to maintain hydration (consumed 500 ml of water) because dehydration has been associated with reduced resting saliva flow rates (Phillips et al., 2012). Samples were collected 24-hour period before and 24-to-48 hour period after 10 different competition weeks beginning in the pre-conference season (early January) and ending one week after the end of season conference competition (early March). On the weeks (n = 3) in which the team did not compete (bye weeks), samples were taken in the same time intervals (48 hours apart).

Saliva Collection

All saliva samples were collected via unstimulated passive drool (0.75 mL) over a time period of 3 to 5 minutes, a procedure all the subjects were familiar with procedure and informed of all testing procedures. Participants were instructed on the following: 1) refrain from brushing their teeth 30 minutes prior to collections, 2) avoid dairy and foods high in sugar or caffeine content for 20 minutes prior to sample collection, and 3) wait at least

10 minutes after food or drink intake before providing sample to avoid sample dilution. Participants were asked to sit quietly, lean slightly forward, and accumulate saliva in the floor of the mouth. All saliva in the subject's mouth was subsequently swallowed prior to providing a whole saliva sample as a standardized saliva collection procedure. During collection, saliva dribbled through a 5 cm plastic straw into a 2 mL polypropylene cryovial (SalivaBio, Carlsbad, CA). Samples were transported in a cooler to a -20°C freezer as soon as possible, typically within 20 minutes of collection.

Instruments-Tests for Hormonal Assays

On the day of analyses, saliva samples were thawed and centrifuged at 3500 rpm for 15 minutes to remove mucins (Sorvall ST40R Multispeed Centrifuge; Thermo Scientific, Waltham, MA) and the resulting supernatant was stored at -80°C until further assay analysis. Samples were assayed in duplicate for T, C and sIgA. All biomarker concentrations were determined using commercially available indirect enzyme-linked immunosorbent assay (ELISA) kits (Salimetrics, Carlsbad, CA) as measured by an absorbance microplate reader (BioTek x808; BioTek Instruments, Inc., Winooski, VT) in accordance with the manufacturer's instructions without modification (Salimetrics). A separate assay was used for each player with 10 assays per hormone, for 20 assays in total, with samples, standards, and controls run in duplicate for all standards, controls, and samples. The minimal concentration that can be distinguished from 0 with this assay is less than 0.03 nmol/L and 0.2 nmol/L for T and C, respectively. Intra-assay coefficients were 4.33%, 4.20%, and 4.47%, and inter-assay coefficients were 2.15%, 6.47%, and 11.26% for C, T, and sIgA, respectively. Cortisol, T, and sIgA are measured in nmol/L, pmol/L, and ug/mL, respectively.

Research Design

In order to investigate the various contributing factors to changes in biomarkers over a competitive season, in each case, a nested model with two levels of predictor variables were included in the model of fixed effects. Level 1 represents within-person change, and level-2 variables vary between wrestlers. These variables include Level-1 variables of Day (outlined above), Pre-Post, and Travel, and two interaction terms (Day X Pre-post, and Day X Travel). In addition, Time since Wake-Up and Bouts were entered into the model as Level-2 variables, and one cross-level interaction term was examined (i.e., Day X Bouts). Level-1 variables were consistent across individuals, indicating changes within the individual: Day represents the day since the start of the season to correct for uneven intervals between samples, Pre-Post is a categorical variable representing pre-competition sample or post-competition samples, and Travel is a categorical variable representing home (no travel) or away (some travel). The time awake for each salivary sample day and whether pre-competition or post competition was recorded. In addition, whether the competition was at home (no travel) or away (travel) was noted. Finally, the day in the study period (Day) and competition number (Bout) were recorded as to test the influence of wrestling season duration on hormonal and immune functions. At Level 2, Time since Wake-Up approximates diurnal rhythm given the varied times each individual woke up the day of practice, and Bouts (i.e. number of competitions between pre-competition and post-competition samples) varied among the wrestlers. Interaction terms were also included in the models, predicting T, C, T:C ratio, and sIgA as anabolic, catabolic, anabolic:catabolic ratio, and immune function outcomes, respectively.

Statistical Analysis

A comparison alpha level of $p \leq 0.05$ was used to determine statistical significance, and "marginally significant" results constitute p-values of < 0.15. Although the tests do not meet the strict $p \leq 0.05$ cutoff values, given the small number of participants in the current study, we interpret them as potentially statistically significant in magnitude-based research design (Hopkins et al., 2009). In order to conceptualize the change in T, C, T:C ratio, and sIgA over the competitive conference season, a series of hierarchical linear models were utilized. Due to the nested nature of the data (i.e., observations were not independent), the model was partitioned into within-person variance, and between-person variance by wrestler, time (Day) at Level 1, and other variables were analyzed at Level 2 and 3. Given the diurnal nature of both T, C and sIgA, variables related to the time of day the sample was taken, and a time since individuals woke up variable was included in the model. Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC) PROC MIXED models. Time since Wake-Up was included in all models as a better metric of diurnal rhythm (vs. absolute time of day) given the individual variability relation to wake-up time for each individual. All data are reported as mean $\pm$ SD in Table 1.

Table 1: Salivary Biomarker Concentration Data by Week of Competition

Competition	Sample	Sample number	n	Testosterone (pmol/L)	Cortisol (nmol/L)	T:C ratio	Secretory IgA (ug/mL)
Week 1	Pre	1	10	863.29 ± 529.15	11.75 ± 5.97	0.073 ± 0.025	
	Post	2	9	540.90 ± 214.37	6.07 ± 2.47	0.096 ± 0.037	156.26 ± 85.58
Week 2	Pre	3	10	621.42 ± 314.54	10.23 ± 6.50	0.072 ± 0.024	
	Post	4	10	480+39± 127.94	5.13 ± 2.66	0.118 ± 0.072	164.02 ± 101.14
Week 3	Pre	5	10	739.98 ± 434.28	10.53 ± 9.17	0.082 ± 0.025	
	Post	6	8	420.92 ± 152.27	4.61 ± 2.40	0.100 ± 0.034	264.49 ± 242.63
Week 4 (Bye)	Pre	7	10	724.88 ± 353.26	8.19 ± 3.21	0.091 ± 0.028	
	Post	8	10	900.18 ± 386.58	8.57 ± 3.12	0.111 ± 0.047	260.79 ± 169.04
Week 5	Pre	9	7	627.08 ± 254.49	7.86 ± 4.24	0.097 ± 0.045	
	Post	10	10	456.43 ± 159.76	5.51 ± 3.84	0.098 ± 0.033	126.94 ± 131.33
Week 6	Pre	11	10	541.23 ± 248.74	5.75 ± 3.75	0.114 ± 0.059	
	Post	12	10	831.69 ± 326.04	13.54 ± 9.04	0.124 ± 0.144	192.41 ± 121.69
Week 7 (Bye)	Pre	13	9	610.01 ± 280.26	7.29 ± 3.58	0.091 ± 0.038	
	Post	14	10	771.34 ± 245.27	11.51 ± 6.46	0.094 ± 0.067	334.51 ± 333.97
Week 8 (Bye)	Pre	15	10	467.86 ± 209.86	4.46 ± 2.56	0.123 ± 0.051	
	Post	16	10	623.10 ± 299.47	6.92 ± 3.63	0.103 ± 0.051	213.56 ± 109.39
Week 9	Pre	17	9	699.27 ± 320.90	8.26 ± 4.02	0.093 ± 0.036	
	Post	18	9	573.12 ± 139.83	4.81 ± 1.98	0.139 ± 0.062	207.96 ± 132.72
Week 10 (Bye)	Pre	19	10	628.77 ± 279.07	6.16 ± 2.73	0.108 ± 0.035	
	Post	20	9	443.32 ± 255.04	5.88 ± 3.67	0.090 ± 0.044	176.24 ± 99.24
Note: Salivary biomarker numbers represent mean values ± standard deviation							

RESULTS

Throughout the conference season and post-season tournaments, not all subjects' performance contributed equally as to the outcome of the match. Some parameters such as individual and team scoring efficiency or competition against national ranking per weight class could influence hormonal and perceptional performance changes. Only team results and average salivary values are reported within this investigation (Table 1).

Testosterone and Cortisol Concentrations

In order to assess the patterns of T and C separately over the conference competitive and tournament season, two hierarchical linear models were conducted. The T model showed evidence of nesting (i.e., lack of independence of observations in T concentrations), $ICC = 0.43$, $p = 0.02$. Table 2 shows the values of the estimates for fixed effects estimates on T levels. In both models, T values were significantly different between wrestlers (see Table 2), $p < .0001$. A full model with eight predictor variables (five individual variables and three interaction terms) were entered into the model. At Level 1, Pre-Post was a significant positive predictor, indicating that on average, the post-competition levels were higher than the pre-competition levels. However, in contrast to the positive Pre-Post effect, Day X Pre-Post indicated that on average, as the season went on, average T levels decreased in post-match samples. There were marginally significant changes over time (Day), but no significant changes between home and away matches (Travel), nor the interaction between the two variables (Day X Travel). As expected, Level-2 variables of time since wake-up and number of bouts were significant negative predictors of T concentrations, indicating that the further from wake-up and as the number of bouts increased, respectively, the T concentrations decreased; for example, for each week where bouts increase by n = 1, testosterone concentrations decreased by 293.57 units (on average), as indicated by the parameter estimates in Table 2. Interestingly, the Day X Bout interaction was a significant positive predictor of T levels, indicating that on average, as the season went on (increase in days), an increase in bouts resulted in an average increase in T over time.

Table 2. Effects on Salivary Testosterone Concentrations

	Empty Model		*Full Model*	
	PE (SE)	*p*	PE (SE)	*p*
Intercept (Wrestler)	641.70 (67.88)	< .0001*	818.92 (78.36)	< .0001*
Fixed Effects	--	--		
Level-1 (Within-Subjects)	--	--		
Day			- 1.62 (1.08)	.13†
Pre-Post	--	--	317.88 (103.25)	.002*
Travel	--	--	168.40 (140.34)	.23
Day X Pre-Post	--	--	-5.40 (2.22)	.02*
Day X Travel	--	--	-7.19 (6.19)	.25
Level-2 (Between-Subjects)	--	--		
Wake-Up	--	--	-0.37 (0.09)	< .0001*
Bouts	--	--	-293.57 (67.31)	< .0001*
Day X Bouts (Cross-Level Interaction)	--	--	5.74 (1.68)	.0008*
Fit Statistics				
-2 Log Likelihood	2635.20	--	2575.70	--
AIC	2641.20	--	2597.70	--
BIC	2642.20	--	2601.00	--

Note: *Statistically significant at p < .05; † Marginally significant at p < .15; PE = parameter estimate; SE = standard error

Cortisol concentrations showed similar nesting, *ICC* = 0.14, *p* = 0.05. Therefore, a full model was also run on C concentrations. There were significant differences in C concentrations between wrestlers (*p* < .0001). At Level 1, unlike T, Day was a significant negative predictors of C concentrations, indicating as the season went on, C decreased. Pre-Post and its interaction with time (Day X Pre-Post) were non-significant. Travel was a marginally significant positive predictor (*p* = 0.11), i.e., cortisol concentrations increased by an average of 4.61 units after travel compared to pre-travel levels (see Table 3 for parameter estimates). However, Day X Travel indicated that as the season progressed, C significantly decreased on average on travel days (see Figure 1 and Table 3). At Level 2, as expected, and in line with T concentrations, time since wake-up and number of bouts were negative predictors of C concentrations. This indicates that as time since wake-up and number of bouts increased, respectively, the C concentrations decreased. Day X Bout showed that as the season progressed, on average, an increase in bouts resulted in an average increase in C over time.

Table 3. Effects of on Salivary Cortisol Concentrations

	Empty Model		*Full Model*	
	PE (SE)	*p*	PE (SE)	*p*
Intercept (Wrestler)	7.89 (0.71)	< .0001*	12.26 (1.12)	< .0001*
Fixed Effects	--	--		
Level-1 (Within-Subjects)	--	--		
Day	--	--	-0.04 (0.02)	.05*
Pre-Post	--	--	1.19 (2.11)	.57
Travel	--	--	4.61 (2.87)	.11†
Day X Pre-Post	--	--	-0.008 (0.05)	.86
Day X Travel	--	--	-0.26 (0.13)	.04*
Level-2 (Between-Subjects)	--	--		
Wake-Up	--	--	-0.009 (0.002)	< .0001*
Bouts	--	--	-3.24 (1.37)	.02*
Day X Bouts (Cross-Level Interaction)	--	--	0.08 (0.03)	.01*
Fit Statistics				
-2 Log Likelihood	1151.60	--	1100.70	--
AIC	1157.60	--	1122.70	--
BIC	1158.50	--	1126.10	--

Note: *Statistically significant at p < .05; † Marginally significant at p < .15; PE = parameter estimate; SE = standard error

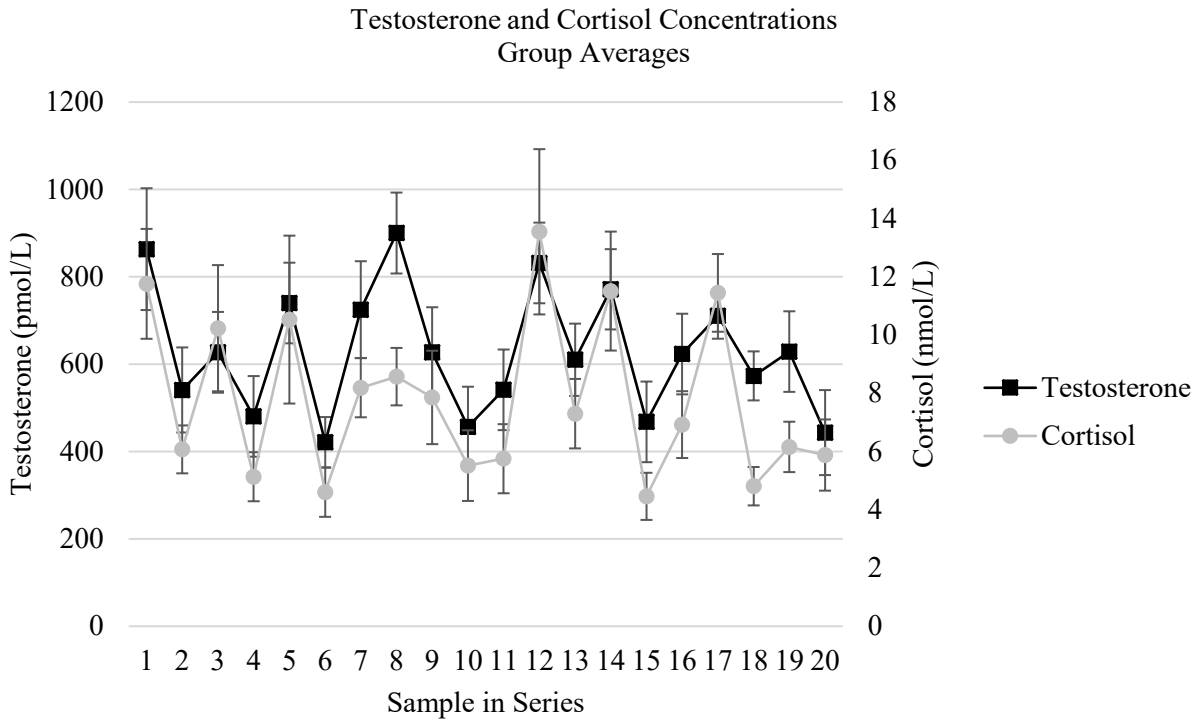

Figure 1: Testosterone and Cortisol Concentrations by Sample

Note: Error bars represent standard error values; sample numbers are shown in Table 1.

Testosterone:Cortisol Ratio

Given some similar and some disparate findings in predictors of T and C concentrations separately, T:C ratio was subsequently analyzed. The initial empty model showed the presence of nesting of T:C ratio values, *ICC* = 0.19, $p = 0.03$, and indicated that Day was a marginally significant predictor of T:C ratio. (Figure 2 for T:C ratios) (Hopkins et al., 2009). The full model with five predictors and three interaction terms showed only two marginally significant predictors and one interaction term, Day X Pre-Post. Interestingly, although Day ($p = 0.07$) and Pre-Post ($p = 0.03$) indicated marginally significantly increased T:C ratios, the interaction term indicated that as days increased, T:C ratios were lowered by an average of 0.0009 units after competitions as compared to pre-competition levels. This is perhaps not surprising given the evidence that only four of the eight predictors overlapped in significantly predicting both T and C concentrations individually (see Table 4 for detailed model results).

Table 4. Effects on Testosterone-to-Cortisol Ratio

	Empty Model		*Full Model*	
	PE (SE)	*p*	PE (SE)	*p*
Intercept (Wrestler)	0.10 (0.008)	< .0001	0.07 (0.01)	< .0001*
Fixed Effects				
Level-1 (Within-Subjects)				
Day	--	--	0.0004 (0.0002)	.07†
Pre-Post	--	--	0.04 (0.02)	.07†
Travel	--	--	0.03 (0.03)	.37
Day X Pre-Post	--	--	-0.0009 (0.0005)	.09†
Day X Travel	--	--	-0.0009 (0.001)	.53
Level-2 (Between-Subjects)				
Wake-Up	--	--	0.00002 (0.00002)	.37
Bouts	--	--	-0.02 (0.02)	.27
Day X Bouts (Cross-Level Interaction)	--	--	0.0005 (0.0004)	.20
Fit Statistics				
-2 Log Likelihood	-581.00		-592.70	--
AIC	-575.00	--	-570.70	--
BIC	-574.10	--	-567.40	--

Note: *Statistically significant at p < .05; † Marginally significant at p < .15; PE = parameter estimate; SE = standard error

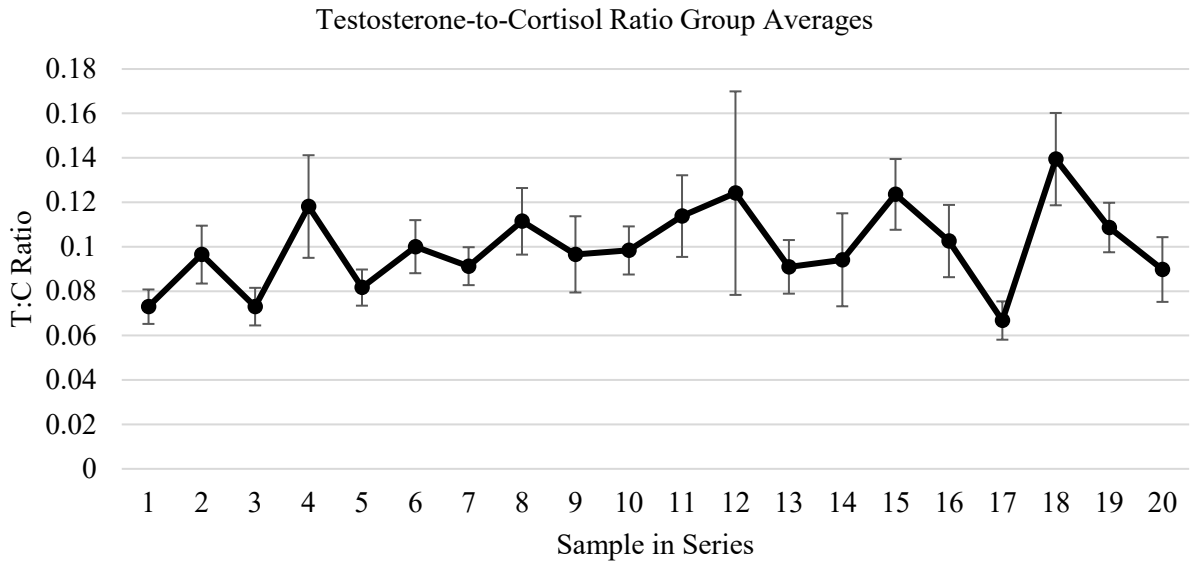

Figure 2: Testosterone:Cortisol Ratio by Sample
Note: Error bars represent standard error values; sample numbers are shown in Table 1.

Secretory IgA

The initial empty model showed the presence of nesting of sIgA values (ug/mL), *ICC* = 0.19, *p* = 0.059. A nested model with all predictors except Pre-Post and its interaction with Day were included because sIgA samples were collected one time per week (all post-match). Sectory IgA concentration levels were significantly different between wrestlers ($p < .0001$). At Level 1 and Level 2, Day, Wake-Up, Travel, and the Day X Travel predictors were non-significant predictors of sIgA levels. The number of bouts in a given week was a significant negative predictor of sIgA concentrations; however, its interaction with time (Day X Bouts) showed a marginally significant positive prediction, indicating that over time as bouts increased, sIgA concentrations increased on average by 1.86 units (Figure 3 for sIgA concentrations and Table 5 for model results).

To summarize the conclusions of this investigation, analyses revealed that Day predicted T, C, and T:C; however, where T and C concentrations decreased over time, T:C ratio increased on average. Pre-Post positively predicted T and T:C ratio, indicating the post-competition levels were higher than the pre-competition levels. In both T and T:C ratio, however, Day X Pre-Post was a significant negative predictor, and C concentrations did not change significantly pre-post, nor over time (Day X Pre-Post).. Bouts negatively predicted T, C, and sIgA concentrations; however, it did not predict T:C ratio, and interestingly, these values increased as the season went on (Day X Bouts). Travel only affected C concentrations, showing increased levels on days traveled; however, C concentrations decreased as the season went on for travel weeks (Day X Travel). The exploration of these salivary biomarkers could prove as objective non-invasive method to understand demands of intense competitive exposures.

Table 5. Effects on Salivary Secretory IgA Levels

	Empty Model		*Full Model*	
Intercept (Wrestler)	210.84 (28.43)	< .0001*	315.76 (75.02)	.002*
Fixed Effects				
Level-1 (Within-Subjects)				
Day	--	--	-1.28 (1.25)	.31
Travel	--	--	86.37 (89.63)	.34
Day X Travel	--	--	-3.33 (4.02)	.41
Level-2 (Between-Subjects)				
Wake-Up	--	--	-0.09 (0.08)	.26
Bouts	--	--	-88.78 (42.84)	.04*
Day X Bouts (Cross-Level Interaction)	--	--	1.86 (1.08)	.09†
Fit Statistics				
-2 Log Likelihood	1291.10	--	1232.50	--
AIC	1297.10	--	1250.50	--
BIC	1298.00	--	1253.20	--

Note: *Statistically significant at p < .05; † Marginally significant at p < .15; PE = parameter estimate; SE = standard error

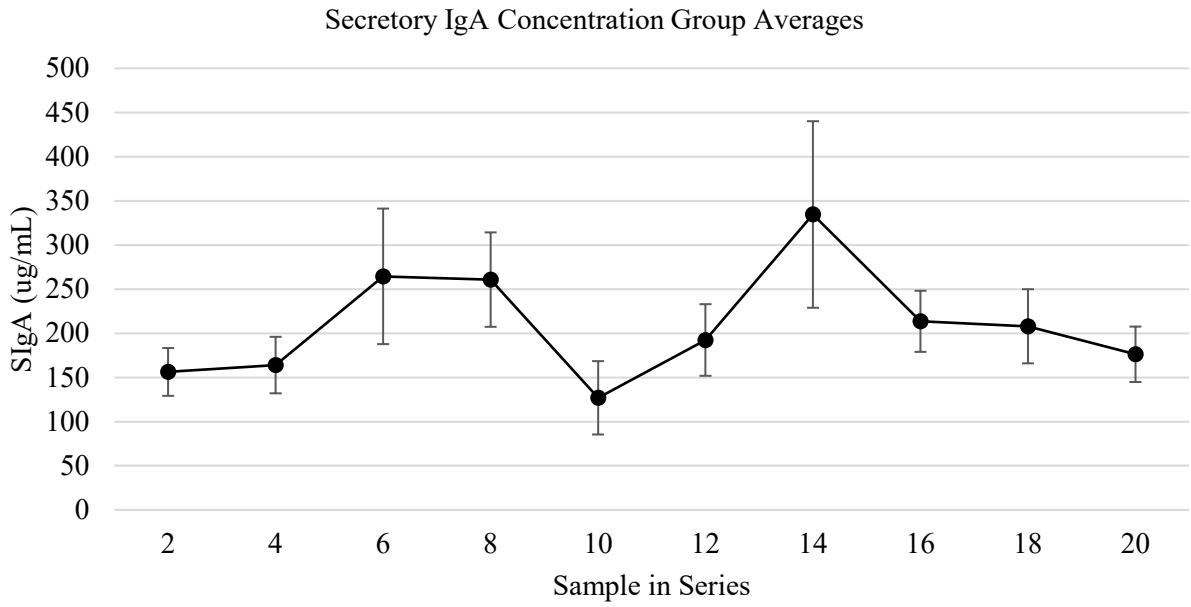

Figure 3: Secretory IgA by Sample
Note: Error bars represent standard error values; sample numbers are shown in Table 1.

DISCUSSION

Salivary analyses have evolved into a sophisticated science and recognized in a variety of biomedical basic and clinical sciences. Saliva is an easily available specimen containing a large array of hormones and enzymes used in screening and diagnostic assessment (Brownlee et al., 2005). Salivary collection is a minimal invasive process compared to current methods of blood collection. The procedures to collect and analyze blood samples can often be expensive, painful and invasive. Yet, investigations on the reliability and validity of salivary analysis to blood-based biomarkers in response to stress remains unclear. Implementing a system of exercise training and periodization that incorporates the use of non-invasive methods allowing for an accurate evaluation of exercise-induced physiological and psychological stress is important. This was the first study to examine and quantify the relationships between hormonal and enzyme variables that surround a typical competitive wrestling season and objective markers of training (T, C, T:C ratio, and sIgA) applied to predict exercise intensity in elite athletes with variable outcomes (Gatti & De Palo, 2011).

Wrestling conference season involves weekly home or away dual meets against high quality teams. While the rigors of each match are less than a single day tournament, the preparation for the competition (i.e. daily training, weight reduction, travel) are similar and can challenge the athlete's ability to cope with stress. During the ten weeks of sample collection, there was evidence of increases in T and decreases in C although the T:C ratio was not significantly different. An increase in T would suggest an anabolic condition while the C decrease would indicate a lowering of "catabolic state" associated with the competitive season. Unfortunately, the changes in these individual hormones did not coincide with the T:C ratio which might suggest independence in these biomarkers. An optimal change over time would be to have a rise in the T:C ratio, which would suggest an adaptation favoring the athlete's ability to recover each week from competition. The competitive portion of the wrestling season is designed to test the skills and conditioning of the athlete and prepare them for competition at the national level. While the rigors of daily training are replaced with competition (which includes travel and weigh-in), the mental and physical challenges (particularly in the Big 10 Wrestling Conference), can strain the body and therefore create problems which could result in overtraining. A taper period is introduced at the end of the dual meeting schedule ("bye week") which is designed to rest the athlete as to prevent overtraining. It should be noted, however, that the schedule of competition for this season included a "bye week" after the three weeks of competition (representing approximately five cumulative bouts per person) against three of the top five placed teams in the 2016 NCAA National Championship. This "bye week" did include training but not having to "make weight" should have affected a summative change in T or C. The pre-salivary C concentration for this week was lower than the three-previous week's pre-salivary C levels suggesting less stress. This could be due solely to the lack of weigh-in, which creates a catabolic state due to restriction of food intake. In addition, the post-salivary T concentration during this week produces the highest mean value across the study period. This response might be due to numerous reasons but does provide some indication of an anabolic condition.

Exercise-induced stress provides a similar avenue for the use of salivary biomarkers in prediction and evaluation. These biomarkers offer potential in both an acute assessment of physiological and psychological stress (Nieman & Henson, 1994). Cortisol was elevated by the stressful in-season training camp while T and T:C did

not change suggesting that a 28-day training camp may not cause significant disturbances in hormonal or biochemical stress markers in elite athletes (Hoffman et al., 1999). Although, findings from the present investigation is consistent with previous investigation on professional basketball subjects, increases in tested T did not occur after a taper. The weeks coinciding with recovery or taper during the season did not alter the sIgA group values suggesting immune resistance is not as reactive to brief periods returned training or changes in dietary restrictions.

The exploration of salivary biomarkers could prove as objective non-invasive method to understand demands of intense competitive exposures. Investigations assessing salivary inflammatory and stress responses may identify and help illuminate some validity issues regarding the assessment of salivary stress, inflammatory and immune system biomarkers. In multiple cases, the immune system exhibits adverse change after prolonged and intense exercise exertion lasting more than 90 minutes (Hoffman et al., 1999). Research data on the resting immunity status of athletes and non-athletes is limited and presents a confusing picture at present (Hofman, 2001). For example, the few studies available suggest that the immune system responds differentially to the chronic stress of intensive exercise, which is dependent on fitness, nutritional, and pathological status (Vining & McGinley, 1987).

In conclusion, investigative analyses revealed a significant Pre-Post T and T:C ratio as a significant predictor, indicating the post-competition levels were higher than the pre-competition levels. In this investigation, Pre-Post C and interaction with time (Day X Pre-Post) were not significant. Although, a marginally significant sIgA concentration (Day X Bouts) showed a positive prediction, indicating that over time as bouts increased, sIgA concentration levels increased. In future investigations, the degree to which salivary biomarkers correspond to blood-based markers is unclear in both a resting state and in response to stress. Monitoring salivary biomarkers is recommended to identify excessive stress caused by the natural demands to meet season demands of training and competition. Supporting a need for additional research on salivary indices validating inflammation and stress measures to systemic and pathological mechanisms. Where this is not possible, any extrapolation of findings from endurance athletes to wrestling demands should be made with caution, since the physiological demands of combative training is not necessarily the same as those of endurance training and competition.

This study provided some evidence that factors such as time of day and time awake might play a role in the levels of biochemical markers of stress and immune function. Based on current findings in conjunction with results from the previous studies, a practical approach in assessing the state of recovery, stress and consistency in these factors would be beneficial testing day to day variance. The demands on athletes can be subjective to other factors than practice or competition. For this study, the time awake varied; in future studies, researchers and practitioners should note if naps are taken or there is disruptive sleep in the previous night. While the researcher can ask for recall on each of these variables, some adjustment might need to be made to control for changes in sleep patterns or awake time between days.

Another implication for future research is to evaluate the interdependence of testosterone and cortisol. While a ratio of these two hormones are reported in the literature, our findings do not support this interdependence. If higher cortisol is catabolic to certain tissue, the rationale for lower levels to promote better recovery is under question. If the lower hormonal levels of testosterone are viewed as detrimental to recovery, then how does variance in levels of testosterone across athletes justify this implication? In many cases heavy exercise training promotes an elevation of testosterone while also elevating the levels of cortisol. In effect, this might result in both anabolic condition concurrently with a catabolic state which supports the Fitness-Fatigue Theory (Chiu & Barnes, 2003) for adaptation to the stress imposed and suggesting the use of a T:C ratio needs farther investigation. The goal for in-season surveillance of biochemical markers is to identify potential signs of overtraining which might lead to injury or illness.

Practical Applications

A complete endocrine profile combined with optimal exercise training is an important factor for any athletic performance. This study has provided a description of elite athletes experience with a seasonal surveillance system using salivary-based medians to determine hormonal levels associated with anabolic and catabolic metabolic processes. In addition, a measure of an antibody, such as sIgA, which has been linked to upper respiratory infection during a period of time in which immunosuppression might be present (Neville et al., 2008). During periods of intensive training and high expectations for athletic performance, many individuals are prone to overtraining and therefore should be monitored for signs associated with this condition. Our data suggest that the performance and health care professional can benefit from this investigation that employed a non-invasive procedure for analyses of easy to obtain bodily fluid which carries biological indicators of stress, recovery state, and immune defense.

While the results of this study provides evidence that will impact decisions on the field, a performance and health care professional should adopt a holistic approach for monitoring exercise training and develop questions

towards the use of salivary sampling in our professions and practice. Future investigations in this area will promote a better understanding of the demands the athletes endures during exercise training and competition. Additionally, provide an application for accurate detection of signs of overreaching and overtraining which need to be dealt with before the athlete reaches a point of decay in health.

Disclosure Statement
The authors report no potential conflict of interest.

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