

The Relationship Between Serum and Salivary Cortisol Levels in Response to Different Intensities of Exercise

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Purpose: The effect of exercise intensity on the tracking of serum and salivary cortisol responses was examined in 12 endurance-trained males (maximal oxygen uptake [VO₂max] = 58.2 ± 6.4 mL/kg/min). **Methods:** Subjects rested for 30 min (control) and exercised on a cycle ergometer for 30 min at 40% (low), 60% (moderate), and 80% (high intensity) of VO₂max on separate days. Serum and saliva samples were collected pretrial, immediately posttrial, and 30 min into the recovery period from each trial. **Results:** Cortisol responses increased significantly for both serum (40.4%; $P = .001$) and saliva (170.6%; $P = .007$) only in response to high-intensity exercise. Peak saliva cortisol occurred at 30 min of recovery, whereas peak serum was at the immediate posttrial sampling time point. The association between serum and saliva cortisol across all trials was examined using concordance correlation (R_c) analysis, which accounts for repeated measures. The overall correlation between serum and saliva cortisol levels in all matched samples was significant ($R_c = 0.728$; $P = .001$). The scatter plot revealed that salivary cortisol responses tracked closely to those of serum at lower concentrations, but not as well at higher concentrations. **Conclusions:** Findings suggest salivary measurements of cortisol closely mirror those in the serum and that peak salivary concentrations do not occur until at least 30 min into the recovery from intense exercise.

Keywords: hormones, endocrine, exercise, HPA axis, physical stress

Cortisol levels increase in response to psychological and physical stressors such as life changes, extreme temperatures, negative energy balance, and physical exercise. Most previous exercise studies investigating cortisol responses are in agreement that there is a “threshold intensity” of one’s maximal oxygen uptake (VO₂max) that results in significant elevations in circulating cortisol.^{1,2} These studies measured cortisol responses by assessing concentrations in the serum or plasma of the blood. While this method is accurate and consistent, collection of blood samples is invasive, can be difficult to obtain during the exercise testing

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process, and can also augment the stress response of the subject due to the collection procedure.³ Thus, some researchers have proposed that cortisol concentrations measured in saliva may provide a feasible, accurate, and practical alternative to blood determinations.⁴ Since cortisol is a lipophilic steroid with low molecular weight, the portion of the hormone that is unbound to carrier proteins (ie, free portion ~1–10% of total) can enter the body fluids through passive diffusion. Thus, it is possible to measure these free cortisol levels in all fluids, including saliva.⁵ Since only a small, unbound fraction of the hormone is available to diffuse into the saliva, concentrations are consistently lower than in serum. At rest and in response to exercise, salivary cortisol levels are thought to have a steady and predictable relationship to free and total cortisol levels.⁶ Previous research (basal-resting) studies support this notion, having found correlation coefficients between cortisol in saliva and blood ranging from $r = .71$ to 0.96 .³ In previous studies employing short exercise bouts (30 min) the relationships between the two methods have also been significant, with correlations of $r = .90$ after 30 min of exercise,⁷ and $r = .93$ after 15 min of recovery.⁸

For the reasons noted above, some researchers consider salivary cortisol concentrations a reliable and accurate measure of this hormone in many situations. There is still some controversy, however, surrounding this technique. Due to the passive movement of cortisol from the blood into the saliva, there may be a delayed response in salivary concentrations before accurately reflecting the levels in the blood.⁹ Interestingly, few exercise studies comparing saliva versus blood sampling have used exercise at varying levels of intensity to provoke and compare cortisol responses.⁴ This is a critical aspect to be studied because an exercise intensity threshold exists for blood cortisol levels,¹ and that following high-intensity exercise the peak blood cortisol response can occur during recovery.⁵ Therefore, the aim of this study was to address aspects of the aforementioned issues and investigate the associations between serum and salivary cortisol levels before, immediately afterward, and at 30 min of recovery from three intensities of exercise (low = 40%, moderate = 60%, high = 80% of VO_2max). The intent was to determine if salivary concentrations accurately reflect the blood responses of the body's stress to exercise and to assess the time course for salivary and blood cortisol changes.

Methodology

Subjects

Twelve moderate to highly aerobically trained male subjects (ages 18–30) were recruited for this study. All had trained consistently for a minimum of 3 d/wk for ≥ 60 min/day for the 6 mo before the study. Each subject was informed of the risks of the protocol, signed a statement of informed consent approved by the university's Institutional Review Board, passed a medical and physical examination, and demonstrated a lack of emotional stress (ie, normal scores on a psychological evaluation—REST-Q questionnaire¹⁰). Subject exclusion criteria included a diet chronically low (<50% of daily caloric consumption) in carbohydrates, a history of hormonal disorders, mental illness, smoking, use of anabolic steroids, or chronic nonsteroidal anti-inflammatory drug use.

Study Protocol

Each subject was asked to report to the laboratory on five separate occasions. They were instructed to maintain and control their diet (eucaloric and at least 50% of calories from carbohydrates) over the duration of the study. The subject completed a 3 d diet record before their initial session. The diet was analyzed using the nutrition database on the website of the United States Department of Agriculture¹¹ to determine the macronutrient breakdown. If the subjects' diet did not meet the required average daily carbohydrate consumption, they were given guidance on how to incorporate additional carbohydrate sources into their diet. After they completed another 3 d diet record and the diet was verified, the subjects were allowed to continue. For all experimental sessions, subjects were asked to report to the laboratory 4 h postprandial, having consumed no caffeine or alcohol for the previous 8 h.

The first visit was used for screening and measuring the subjects' VO_2max . The next four visits, on separate days, consisted of a control rest period, and the 30 min exercise bouts at low, moderate, and high intensity. All subjects did not complete the sessions at the same time (between subjects) but all four of the visits (within subjects) were completed at the same time of day (± 30 min). The order of each of the subjects' exercise sessions were randomly assigned and were separated by a minimum of 72 h.

Screening Session. Once at the laboratory the subjects underwent a medical screening process. After approval for participation, anthropometric data (age, height, body mass, body fat percentage via skinfolds) were obtained. They were next instructed to warm up on a cycle ergometer for 5 min at a very light workload (< 50 W) followed by 5 min of stretching. The incremental VO_2max exercise test began at a workload determined by the subjects' training history (either 50 or 100 W). The workloads for the test were increased by 25 or 50 W at the end of each 3 min stage until volitional fatigue. Respiratory gases were collected continuously over 15 s intervals, heart rate (HR) was recorded at the end of every minute, and ratings of perceived exertion (RPE) were assessed at the end of each exercise stage. The test was considered valid and reliable if the subjects met three out of the four of the following criteria: a 0.15 L/min or less increase in VO_2 in response to an increased workload, a HR at the age-predicted maximum ($\pm 5\%$), a respiratory exchange ratio (RER) of 1.1 or greater, and an RPE rating of 18 or greater.¹²

Experimental Trials. Subjects reported to the laboratory on a separate day (same time of day ± 30 min), at least 72 h but no more than 7 d following their VO_2max test. They completed the REST-Q questionnaire and, if normal scores were demonstrated, they began their supine resting period; if not, they were excused from testing that day and rescheduled. After a 30 min rest, a preexercise blood sample was obtained with the subject still in the supine position and a saliva sample was taken. Next, the subject began a 5 min warm-up on the cycle ergometer at very low intensity (approximately 15–20% of VO_2max) followed by 5 min of stretching. They then mounted the cycle ergometer and began the exercise trial at the predetermined workload to elicit either 40% (low), 60% (moderate), or 80% (high intensity) of their VO_2max . Heart rate was monitored every 5 min, and respiratory gases were collected for 3 min periods at minutes 7–10, 17–20, and 27–30. After the 30 min of exercise was completed, the immediate postexercise blood and saliva

samples were collected. Subjects were then allowed to actively cool down on the cycle ergometer for 5 min, followed by a passive recovery period (30 min from the end of exercise). After the recovery period, the last blood and saliva samples were collected.

This process was replicated for the three exercise intensities. For the resting or control trial, the above procedures were repeated, except a 30 min supine rest was completed by the subjects.

Instrumentation. The height (cm) and body mass (kg) of each of the subjects were determined using a stadiometer (Perspectives Enterprises, Portage, MI) and a mechanical scale (Detecto, Webb City, MO), respectively. Body fat percentage was estimated with skinfolds measured in triplicate at three sites (abdomen, chest, and thigh) using Cambridge Lange skinfold calipers (Cambridge Scientific, Cambridge, MA) and calculated using Jackson-Pollock equation.^{12–14} Body fat was assessed in order to physically characterize the subjects. Respiratory gases were measured using a Parvo Medics TrueMax 2400 Metabolic System (Parvo Medics, Salt Lake City, UT, USA) and all exercise trials were completed on a Lode electronically braked cycle ergometer (Lode, Groningen, The Netherlands). Heart rate was monitored during the exercise using a Polar HR monitor (Polar Model F1, Finland). Ratings of perceived exertion (RPE) were determined using Borg's 6–20 scale rating of perceived exercise scale.¹⁵

Specimen Procedures. Blood samples were collected using a 22 gauge catheter (Angiocath) placed in a vein at the antecubial fossa of the arm. Blood samples (3 mL) were withdrawn using a 3-cc syringe and a 25 gauge needle. Hematocrit (Hct) was assessed in triplicate using the microhematocrit method. Hemoglobin (Hb) levels were also assessed in triplicate using the Stat-Site, WT-9[™] Hemoglobin Meter (Stanbio Laboratory, Boerne, TX). The Hct and Hb values were used to calculate the changes in plasma volume via the Dill and Costill method.¹⁶ The remaining sample was split and portions immediately transferred into EDTA (Vacutainer) and serum separator tubes (Vacutainer). All tubes were kept on ice until processed. The samples were centrifuged (4°C, 3000 × g) and the plasma or serum was decanted and frozen at –80°C for later analysis.

To collect the saliva samples, subjects rinsed their mouths with water, spit, and then allowed saliva to accumulate in the pool of their mouth. If saliva secretion needed to be stimulated, subjects were asked to chew on paraffin film. Accumulated saliva samples (minimum of 0.5 mL) were collected from the subjects' mouths directly into a polypropylene cup. No more than 5 min past the desired collection time point was allowed to pass before saliva was obtained. Collected saliva samples were transferred into cryo-freeze tubes and stored at –80°C for later analysis.

Cortisol Analysis

Total serum cortisol concentrations were measured using a single-antibody, solid-phase radioimmunoassay technique (Siemens Health Care, USA). The saliva cortisol levels were measured using an expanded range high sensitivity enzyme immunoassay kit (Salimetrics, State College, PA, USA). All serum and saliva specimens were assayed in duplicate and coefficients of variation for within-between assay determinations of 10% or less were required.¹⁷

Data Analysis

Mean and standard deviations for all measures were computed (SPSS version 17.0, LEAD Technologies, Inc., Chicago, IL, USA). Separate, 4×3 (trial \times sampling time), repeated-measures ANOVA tests were used to determine if significant changes occurred in serum and saliva cortisol levels. For significant ANOVA results, Tukey post hoc tests were used to determine which means were significantly different.

To determine the strength of the associations between saliva and serum cortisol values from all the repeated-measures biological samples, concordance correlation (R_c) was determined from a mixed-model analysis (PROC MIXED, SAS, Cary, NC). Mixed model analysis accounts for the repeated observations per subject and provides an overall significance level for weighted group analysis. The concordance correlation (R_c) is similar to a correlation coefficient and has a range of -1 to $+1$, with values closer to unity indicating a strong fit, while values near zero indicating little or no fit. The R_c is deemed more appropriate for within-participant, mixed-model regression analyses.¹⁸ The significance level was set a priori at $\alpha \leq .05$.

Results

The physical characteristics of the 12 subjects were as follows: age = 22 ± 5 y; height = 174.9 ± 7.1 cm; mass = 66.5 ± 9.5 kg; BMI = 21.6 ± 1.9 kg/m²; and body fat = $8.4 \pm 2.1\%$. The VO_2max of the subjects was 58.2 ± 6.4 mL/kg/min (see Table 1 for detailed test results). The subjects' mean CHO intake before and during the study was $58\% \pm 4\%$ and all days of diet analysis were isocaloric.

The results of the exercise trials indicate that the actual intensities achieved were slightly higher than desired ($\approx 5\%$), but did not overlap one another (see Table 2). The HR, VO_2 , and RPE responses were significantly ($P = .01$) greater with each increasing intensity for the three exercise bouts (Table 2).

Percent changes in plasma volume ($\Delta\%PV$) were calculated from pre- to immediate posttrial, as well as from pretrial to 30 min into recovery. The respective $\Delta\%PV$ changes were (pre- vs immediate posttrial; pre- vs 30 min recovery): control = -6.1 ± 3.3 , -4.2 ± 2.0 ; low = -11.1 ± 4.2 , -5.1 ± 4.4 ; moderate = -10.4 ± 4.4 , -6.1 ± 5.0 ; high = -14.2 ± 5.6 , -8.0 ± 4.0 . All exercise trials resulted in a significantly greater ($P < .05$) reduction in $\Delta\%PV$ pre- to immediate posttrial

Table 1 Mean (\pm SD) values of the VO_2max test for the subjects ($n = 12$)

Measure	Value
Absolute VO_2max (L/min)	3.84 ± 0.43
Relative VO_2max (mL/kg/min)	58.2 ± 6.4
Peak Heart Rate (bpm)	194 ± 6
Peak RER	1.09 ± 0.04
Peak RPE	19 ± 1
Length of Test (min)	15.6 ± 2.0

Table 2 Experimental test session results (mean \pm SD; average of 30 min of exercise) of select variables measured during each exercise trial ($n = 12$)

Measure	Low Intensity (40% VO ₂ max)	Moderate Intensity (60% VO ₂ max)	High Intensity (80% VO ₂ max)
Workload (W)	95.0 \pm 17.0	145.4 \pm 18.8	181.9 \pm 26.1
Heart Rate (bpm)	127 \pm 14	156 \pm 14	180 \pm 8
VO ₂ (mL/kg/min)	26.3 \pm 3.3	37.8 \pm 4.3	49.4 \pm 4.6
% VO ₂ max	45.3 \pm 5.0	65.2 \pm 7.7	85.0 \pm 5.8
RPE	10 \pm 2	13 \pm 2	16 \pm 1

than what was found for the control trials. No other significant changes in $\Delta\%PV$ between the trials were noted.

Cortisol Responses

The serum and salivary cortisol responses to the different trials at the three respective sampling time points are in Table 3. These hormonal values are reported not corrected for $\Delta\%PV$ changes as uncorrected values reflect *in vivo veritas* (ie, actual natural scenario), and this approach has been recommended in the literature.⁵ During the control and low-intensity exercise trials the serum cortisol concentrations did not change significantly. At moderate intensity exercise, serum cortisol did not change from pretrial to immediate posttrial; however, from immediate posttrial to 30 min into recovery cortisol decreased by 31.2% ($P = .006$). For the high-intensity exercise, serum cortisol increased significantly from pretrial (40.4%; $P = .001$) to immediate posttrial, as well from pretrial to 30 min of recovery (40.4%; $P = .001$). For the control, low-, and moderate-intensity exercise trials, salivary cortisol did not change significantly. During the high-intensity exercise, salivary cortisol responses increased significantly from pretrial to immediate posttrial (105.3%; $P = .005$) and also from pretrial to 30 min of recovery (170.6%; $P = .007$).

Figure 1 displays the scatter plot of the relationship between serum and saliva cortisol at all sampling times within all the trials combined. Different levels of regression analysis were employed, yet the linear regression was the best fit for the distribution of the data. The correlation between these serum and saliva cortisol values was $R_c = 0.728$, which was highly significant ($P = .001$).

Discussion

The primary purpose of this study was to investigate the associations between serum and salivary cortisol responses at three different exercise intensities (40%, 60%, and 80% of VO₂max), to determine if salivary concentrations could be an accurate method to quantify the body's response to the stress of exercise. Each of the exercise trials produced the desired physiological responses; ie, increasing levels of physical stress. This was apparent when looking at the VO₂, HR, and RPE values of the subjects during the exercise trials.^{13,14}

Table 3 Mean cortisol concentrations (mean \pm SD) for each respective experimental trial ($n = 12$)

Trial	Sample	Cortisol Concentrations ($\mu\text{g/dL}$)		
		Pretrial	Posttrial	30 min into Recovery
Control	Serum	12.2 \pm 5.4	9.8 \pm 4.3	10.5 \pm 4.9
	Saliva	0.256 \pm 0.168	0.251 \pm 0.148	0.224 \pm 0.200
Low-Intensity Exercise	Serum	14.3 \pm 7.0	13.8 \pm 6.0	10.9 \pm 4.3
	Saliva	0.268 \pm 0.204	0.213 \pm 0.157	0.190 \pm 0.138
Moderate-Intensity Exercise	Serum	14.0 \pm 4.5	16.2 \pm 5.1	11.1 \pm 5.1**
	Saliva	0.189 \pm 0.114	0.293 \pm 0.133	0.259 \pm 0.165
High-Intensity Exercise	Serum	12.2 \pm 4.4	20.5 \pm 6.7*	20.5 \pm 7.8*
	Saliva	0.170 \pm 0.073	0.349 \pm 0.216*	0.460 \pm 0.251*

*Significant difference from respective pretrial ($P < .05$).

**Significant difference from respective immediate posttrial ($P < .05$).

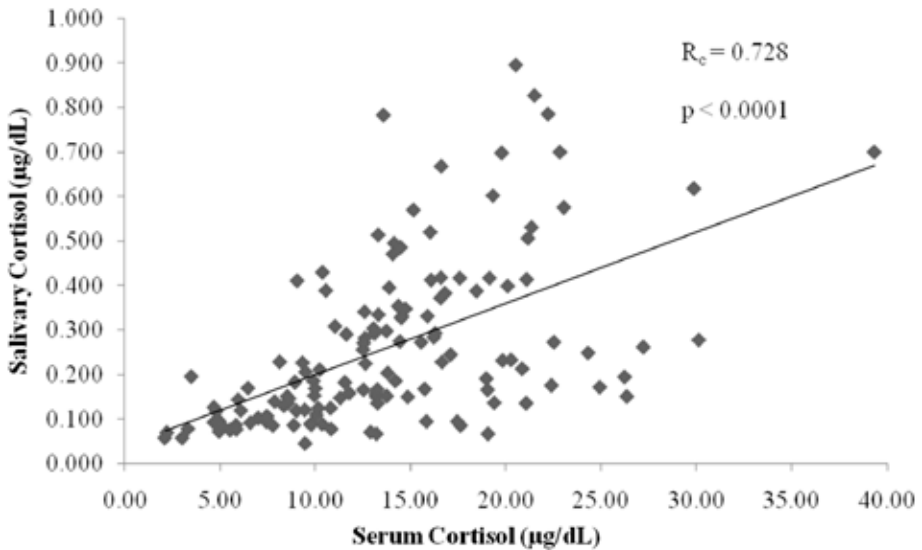


Figure 1 — The relationship between matched serum and salivary cortisol responses across all experimental conditions and time intervals.

Serum cortisol values for all trials were within normal expected range of blood cortisol (Siemens Health Care, USA). Similarly, all saliva cortisol values were within the normal expected range of cortisol and represented between 1 and 10% of the values found in the blood (Salimetrics, USA). Both serum and salivary hormonal concentrations displayed anticipated circadian variations across the course of the sampling times within each trial.^{5,19} Ideally, we would have standardized the time of day for all testing to minimize circadian influences (eg, midafternoon).⁵ However, our subjects each performed their exercise trials at the same time of day, but each person's start time varied due to their work, school, or personal obligations.

High-intensity exercise was the only level to evoke a significant increase in serum cortisol (ie, at immediate posttrial and 30 min into recovery). It had been expected that the moderate-intensity exercise would cause significance increases, except to a magnitude lower than that of the high-intensity trial. The lack of an increase in the blood cortisol levels in response to the moderate-intensity trial most likely is a function of the high aerobic fitness of our subjects. That is, Bloom et al²⁰ and Viru et al²¹ have reported that persons who are highly trained tend to have a somewhat higher intensity threshold to provoke significant increases in circulating blood cortisol.

Saliva cortisol responses to the exercise trials mirrored those of serum. Only in response to the highest-intensity exercise were significant increases above pretrial levels noted, and these increases were at the immediate posttrial and at 30 min into recovery (ie, as with serum responses). This finding is a logical outcome, since blood cortisol levels and changes are what drive the salivary hormonal response.

The correlation between all serum and saliva cortisol was 0.728, which was highly significant and the effect size is considered to be “large” to “very large” in magnitude.²² This correlation is in agreement with other published findings, but is slightly lower than some previous reports. For example, Kirschbaum and colleagues found correlation coefficients between cortisol in saliva and serum ranging from $r = .71$ to $r = .96$.³ Umeda and colleagues reported a correlation of $r = .89$ between serum and saliva cortisol.⁹ Similarly, Goodyer and colleagues found a correlation of $r = .81$.²³

However, it is important to note these studies utilized Pearson product-moment correlation analysis in their statistical approaches. The use of the Pearson analysis does not appropriately account for the repeated measurement effect within designs as we employed here, and employed by these other studies, thus potentially leading to spurious results.¹⁸ Our use of the concordance correlation analysis corrects for this problem and makes our approach in this study unique and more valid within this type of repeated-measures research design.¹⁸

Inspection of the scatter plot (Figure 1) reveals that the association of saliva and serum values appears quite robust at lower concentrations as compared with the higher concentrations. The highest saliva and serum concentrations of cortisol occurred after the high-intensity exercise trial. The lack of a stronger association between saliva and serum at these higher concentrations may reflect a diffusion rate difference between the saliva and serum as blood concentrations of cortisol become elevated. This point is evident by the highest salivary cortisol response occurring at 30 min into recovery whereas the peak serum response occurred immediately after exercise. It should be noted that the peak salivary response after the high-intensity exercise may not accurately reflect true peak responses since levels were only assessed after 30 min of recovery. The salivary responses were still increasing after 30 min of recovery and may have been even higher if we had assessed at a later recovery time point. It is possible the delayed saliva response may be due to the lack of blood flow to the mouth (ie, salivary glands) during high-intensity exercise, because of the blood being shunted more toward the working skeletal muscle during exercise. The reduced blood flow at the mouth could decrease the cortisol available to cross from the capillaries to the saliva through the passive diffusion process. However, once the exercise is complete, the blood is steadily redistributed from the exercising muscle to the rest of the body, including the mouth.^{13,14} Thus, during recovery from exercise the amount of blood flow to the mouth would slowly return to normal or even be enhanced, thereby increasing the amount of free cortisol available to diffuse from the blood into the saliva.

It is interesting to note that the relative magnitude of the peak cortisol responses for salivary and serum did not agree completely (ie, saliva tended to be greater percent change than serum). At first this would appear paradoxical, but it is important to remember that salivary cortisol is a function of the free cortisol levels in the blood. The free hormone levels are not only strongly related to the total amount of the hormone in the serum, but also to the level of binding between the free hormone and its carrier proteins. Fahrner and Hackney have demonstrated that exercise alters the binding affinity of steroid hormone carrier proteins, thereby increasing the amount of free hormone available during and after exercise.²⁴ Such an occurrence would explain the incongruent change in the magnitudes of peak cortisol increases in response to exercise observed here.

Practical Applications

There are several limitations to the current investigation that may impact the findings and interpretations of the data. First, the small sample size and the physical activity demographics of the subjects (endurance trained male athletes 18–30 y of age) make it difficult to generalize the findings to other populations. Although there were only 12 subjects, we had sufficient power ($\beta = 0.8$; $\alpha = .05$) to detect differences in salivary cortisol of $0.2 \mu\text{g/dL}$ and serum cortisol of $0.5 \mu\text{g/dL}$. Second, some subjects completed their trials in the morning whereas others completed their trials in the afternoon, potentially causing differing responses between subjects, although each respective subject did consistently replicate the time of day for each trial. Third, the sampling procedure for saliva could have affected results. Schwartz and Granger²⁵ report the presence of blood in saliva due to oral lesions can affect salivary assay results. Our subjects did not have their mouths inspected before saliva collections to determine if this could have been a problem. It should be noted, however, that no salivary cortisol values were found to be outside of the normal clinical range, which would be expected with blood contamination occurrences. Finally, this study did not include additional recovery sampling points beyond 30 min, which may have been necessary to assess the time course for when the true peak cortisol responses occurred. Conversely though, this study is strengthened by the rigidly enforced experimental controls on diet, other physical activity, external stressors, and standardized exercise sessions, as well as highly specific analytical procedures for cortisol determination. These experimental controls taken collectively reinforce the validity and reliability of the outcomes.

As the saliva cortisol measurement method becomes more established, it may prove to be beneficial to the field of sports physiology. Assessing salivary cortisol allows for the opportunity to collect the samples without medical personnel, it minimizes stress in the collection process, permits frequent and rapid sampling, and facilitates sampling in a variety of environments.²⁵ For a research scientist studying responses of this hormone to exercise, it would enable more data to be collected (more frequent samples and more total samples over a longer period of time) since the process of saliva sampling is much less invasive and time consuming compared with drawing blood. Furthermore, athletes and coaches may be more agreeable and willing to provide samples for analysis without having to disrupt a training or competition schedule with frequent visits to a laboratory. In addition, samples collected outside the laboratory to be later analyzed for salivary cortisol concentrations may more truly reflect training and competition environments; therefore, conclusions drawn from the results may have more external validity. Monitoring levels of this hormone throughout and between training cycles may lend insight into the stress that a training program provides. This may help in the exercise prescription process to facilitate the optimal training stimuli to promote adaptation to training and prevent elevated cortisol responses over an extended period of time (ie, the latter has been associated with the occurrence of the overtraining syndrome).^{14,19,26} Through proper adjustments to the volume, duration, and intensity of training or the incorporation of additional recovery, it could promote maintenance of stable and optimal cortisol responses.

Conclusions

In conclusion, this is one of the few studies to systematically compare serum and saliva cortisol levels at rest and in response to varying exercise intensities and into the recovery from exercise. Collectively, the results suggest salivary measurements of cortisol can, in some circumstances, closely mirror those in the serum in response to exercise. However, the present data do not support the notion that salivary and serum cortisol changes track one another in a temporal manner exactly in response to exercise. This seems specifically evident when blood levels of cortisol reach higher concentrations, which occurs in response to intensive exercise. Thus, our data support the practice that to obtain peak salivary cortisol responses to high-intensity exercise, samples should be obtained at least 30 min into the recovery process.

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