

Salivary and serum cortisol levels during recovery from intense exercise and prolonged, moderate exercise

AUTHORS: Powell J, DiLeo T, Roberge R, Coca A, Kim J-H

Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory, Pittsburgh, PA, USA

ABSTRACT: The aim of this study was to compare serum (SERc) and salivary cortisol (SALc) responses during recovery from two different exhaustive exercises to determine peak cortisol sampling time and the agreement between SERc and SALc levels. Twelve healthy men underwent a maximal treadmill graded exercise to exhaustion (MEx) and a prolonged, submaximal cycle exercise in the heat for 90 min (PEX) while SERc and SALc samples were taken in parallel at baseline, end of exercise, and 15 min intervals over one hour of recovery. MEx and PEX significantly increased SERc and SALc levels ($p < 0.01$) while absolute SERc levels were approximately 7-10 folds higher than SALc. SERc and SALc showed highly positive correlation ($R = 0.667-0.910$, $p < 0.05$) at most sampling times and only a few individual values were out of 95% limit of agreement when analyzed by Bland-Altman plots. However, peak SERc levels (MEx: 784.0 ± 147 , PEX: 705.5 ± 212.0 nmol \cdot L⁻¹) occurred at 15 min of recovery, whereas peak SALc levels (MEx: 102.7 ± 46.4 , PEX: 95.7 ± 40.9 nmol \cdot L⁻¹) were achieved at the end of exercise in MEx and PEX. The recovery trend of SERc and SALc also differed following MEx and PEX. Activity of 11 β -hydroxysteroid dehydrogenase type 2 enzymes may be suppressed following MEx compared to PEX. In conclusion, sampling for peak SERc and SALc levels should take into account their evolution and clearance characteristics as well as type of exercise performed, whereas SALc appeared to be a more sensitive marker than SERc for the measurement of cortisol responses during exercise recovery.

CITATION: Powell J, DiLeo T, Roberge R, Coca A, Kim J-H. Salivary and serum cortisol levels during recovery from intense exercise and prolonged, moderate exercise. *Biol Sport*. 2015;32(2):91-95.

Received: 2014-07-10; Reviewed: 2014-07-19; Re-submitted: 2014-09-16; Accepted: 2014-10-09; Published: 2015-01-14.

Corresponding author:

Jung-Hyun Kim

Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory, Pittsburgh, PA, USA
E-mail: inr3@cdc.gov

Key words:

cortisol
saliva
hormonal marker
heat stress
recovery
exercise
young adults
male

INTRODUCTION

Cortisol (COR), a glucocorticoid hormone, has gained prominence over several decades as a biomarker of stress from physical or psychological stimuli. COR measurements have been widely implemented to assess physical stress response to exercise, training, or comparative events in sports science and to work or environmental stress in occupational physiology and epidemiology investigations [1, 2].

Among laboratory tests for COR, 24h-urinary free COR has been suggested as a gold standard that accurately represents the overall bodily production of COR [3], which is however a time-consuming process and dependent upon a subject's (or patient's) cooperation. Serum COR (SERc) is invasive, stress inducing, and requires blood handling procedures that may not be readily performed in situations involving collection of multiple samples within a narrow time frame or in a field study (e.g., sport, training event).

Salivary COR (SALc) is increasingly utilized because it is non-invasive, painless, and rapidly sampled, making it a more preferred analytical method of COR measurement in sports and occupational research. Numerous studies have validated the practical utility and accuracy of SALc response to physical stress [1]. However, to our

knowledge, no study has compared SALc and SERc at multiple sampling points during an extended period of recovery from exercise. This is important for planning COR sampling from multiple subjects as, for example, following an athletic event and/or occupational activities in which sample collection timing may differ across subjects and possibly confound COR measurements and comparisons. The present study was aimed at comparing SALc and SERc across different sampling times as well as determining the agreement between SALc and SERc during recovery from two different bouts of exhaustive exercises.

MATERIALS AND METHODS

Subjects. Twelve non-smoking, healthy male subjects (age 22.3 ± 2.4 years; weight 77.5 ± 9.7 kg; height 183.5 ± 6.7 cm; VO_{2max} 61.5 ± 4.9 ml \cdot kg⁻¹ \cdot min⁻¹) participated in the present study. All subjects underwent a medical examination and provided written informed consent prior to study participation. The study was reviewed and approved by the NIOSH Human Subjects Review Board and performed in accordance with the ethical standards of the Helsinki Declaration.

Procedure

Subjects arrived at the laboratory between 7:30 and 8:00AM (to control for circadian rhythm influence) [4], while abstaining from caffeine products and strenuous exercise for at least 48 hours. Following a brief medical exam, subjects rinsed their mouth twice with 200 mL of laboratory grade pure water (EMD Millipore Co. USA) to remove saliva and any food debris. After a 15-20 min stabilization, subjects were instrumented with a 22-gauge angiocatheter into an antecubital vein for blood sampling. Thereafter, they placed a sterile cotton swab (Salivette, SARSTEDT AG & Co., Germany) in their mouth, gently chewed it for 1 min to stimulate salivation, and placed the saturated swab into a plastic vial. Concomitantly, 4 mL of venous blood was collected into a blood tube containing a serum-separation activator. After baseline sampling, subjects performed either a maximal treadmill exercise to exhaustion (MEx) using the Bruce protocol [5] or a 90 min cycle exercise (PEX) at 55-60% VO_{2max} in the heat (ambient temperature 45°C, relative humidity 20%) as assigned in a counterbalanced manner. Following exercise completion, subjects recovered for 60 min seated on a chair in a thermoneutral environment, while SALc and SERc sampling occurred immediately at exercise termination and, thereafter, at 15min intervals. Exercise trials were separated by ≥ 7 days for a full recovery from exercise.

Cortisol assays

Saliva and serum samples were centrifuged at 1,000 RPM for 5 min and 3,500 RPM for 10 min, respectively, and stored at -40°C for later analysis via an enzyme-linked immunosorbent method (ALPCO Diagnostics, USA). Standards, controls, and samples were analyzed in duplicate, and sample absorbance was also read in duplicate

using an automated microplate reader (Versa Max, Molecular Devices LLC, USA). The assay sensitivity and intra-assay coefficient of variations were 1.0 ng·dL⁻¹ and 6.5%, and 0.4 μ g·dL⁻¹ and 5.0% for SALc and SERc, respectively. All SALc and SERc values were converted and reported in nmol·L⁻¹.

Statistical analysis

To determine the relationship and agreement between SALc and SERc, Pearson correlation coefficients and 95% limits of agreement (LoA: mean difference \pm 1.96 SD) using Bland-Altman plots were calculated, respectively. Two-factors repeated measures ANOVA was then carried out with Greenhouse-Geisser correction to determine the main effect of condition and time on COR responses. A post-hoc pair-wise comparison was carried out for a significant F-ratio when $p < 0.05$.

RESULTS

One subject did not complete PEX and another was excluded from analysis due to hemolysis of his blood samples. Therefore, 11 (MEx) and 10 (PEX) subjects were analyzed for SALc and SERc, respectively, from which a retrospective power analysis showed a study power of 0.96 (calculated from the mean changes in SALc from the baseline to the end of exercise in PEX: 50.2 \pm 37.6 nmol·L⁻¹, sample size: 10, alpha: 0.05).

Group means (SD), 95% confidence interval of the means, and Pearson correlation at each measurement time are presented in Table 1. SERc and SALc showed a significant positive relationship at each measurement time in MEx and PEX, except at 30 min in MEx.

In the MEx condition, there was a significant main effect of condition ($F = 412.0$, $p < 0.001$) between SALc and SERc and time ($F = 17.3$, $p < 0.001$) on COR response over the course of measure-

TABLE 1. Serum cortisol (SERc) and salivary cortisol (SALc) levels during recovery from graded maximal exercise (MEx) and prolonged exercise in a hot environment (PEX)

Measurement Time	MEx			PEX		
	SERc (nmol·L ⁻¹)	SALc (nmol·L ⁻¹)	R	SERc (nmol·L ⁻¹)	SALc (nmol·L ⁻¹)	R
Baseline	499.4 \pm 135.6 [408.2 - 590.6]	52.0 \pm 27.2 [33.7 - 70.2]	0.755*	477.5 \pm 132.8 [382.4 - 572.5]	45.5 \pm 14.3 [35.2 - 55.7]	0.857*
End-exercise	621.7 \pm 160.4 [513.9 - 729.5]	102.7 \pm 46.4 [74.5 - 133.8]	0.808*	616.4 \pm 138.9 [500.5 - 822.6]	95.7 \pm 40.9 [66.5 - 125.0]	0.827*
15min recovery	784.0 \pm 147.0 [685.3 - 882.8]	90.9 \pm 36.1 [66.6 - 115.2]	0.806*	705.5 \pm 212.0 [553.6 - 856.9]	89.0 \pm 49.6 [53.5 - 124.5]	0.703*
30min recovery	722.1 \pm 131.7 [633.6 - 810.7]	89.4 \pm 35.9 [65.2 - 113.5]	0.590	616.4 \pm 138.9 [516.9 - 715.8]	83.5 \pm 50.4 [47.4 - 119.6]	0.697*
45min recovery	644.0 \pm 103.6 [574.3 - 713.6]	90.1 \pm 49.4 [56.8 - 123.3]	0.667*	533.8 \pm 141.2 [432.8 - 634.9]	75.7 \pm 53.1 [37.7 - 113.8]	0.855*
60min recovery	629.9 \pm 129.9 [542.5 - 717.2]	77.1 \pm 47.5 [45.1 - 109.0]	0.818*	483.6 \pm 137.6 [385.1 - 582.1]	71.9 \pm 42.5 [41.5 - 102.4]	0.910*

Note: Values are mean \pm SD [95% confidence interval: Lower-Upper]. *: R (Pearson correlation) is significant at $p < 0.05$ (two-tailed).

ments. The interaction between the condition and time on COR was also significant ($F=16.6$, $p<0.001$). Bland-Altman plots showed a bias of 566.5 (134.1) [95% CI: 533.5 – 599.4] and LoA of 303.5 – 829.4 between SALc and SERc (Figure 1).

In the PEx condition, there was a significant main effect of condition ($F=183.9$, $p<0.001$) between SALc and SERc and time ($F=9.2$, $p=0.001$) on COR response over the course of the measurements. Interaction between the condition and time on COR was also significant ($F=9.3$, $p=0.001$). Bland-Altman plot showed the bias of 502.7 (153.6) [95% CI: 463.1 – 542.4] and LoA of 201.6 – 803.8 between SALc and SERc (Figure 2).

DISCUSSION

The current study demonstrated that peak SALc levels increased proportionally more than peak SERc levels during MEx (97.5% vs

57%, respectively) and during PEx (110% vs 48%, respectively), but mean SERc levels were quantitatively 7-10 times higher than SALc, as reported previously [6-8]. The increased SALc and SERc levels were significantly correlated ($R=0.667-0.910$) during MEx and PEx at baseline, end of exercise, and during recovery (Table 1). Further analysis of SALc and SERc, via Bland-Altman plots, revealed only small numbers of data points (four and three data points for MEx and PEx, respectively) were out of LoA, but mostly strong agreements between the two measurements across variable sampling times (Figure 1)

The proportionally higher peak SALc levels following MEx and PEx, compared with peak SERc levels, are explained by saturation of the corticosteroid binding globulin sites at high concentrations of COR after exercise that lead to disproportionate increases of serum free and, consequently, SALc that may provide a better measure than

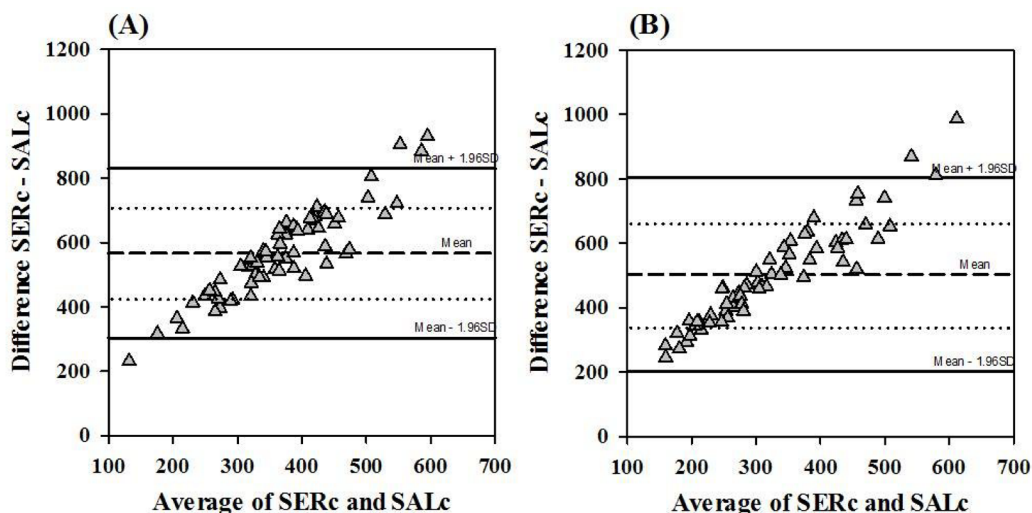


FIG. 1. Bland-Altman plots for SERc and SALc during MEx (A) and PEx (B). Center broken line: mean difference (bias) between the two measurements. Values are in $\text{nmol} \cdot \text{L}^{-1}$. Upper and lower dot lines: mean difference \pm 1 SD. Upper and lower solid lines: mean difference \pm 1.96 SD (95 % LoA).

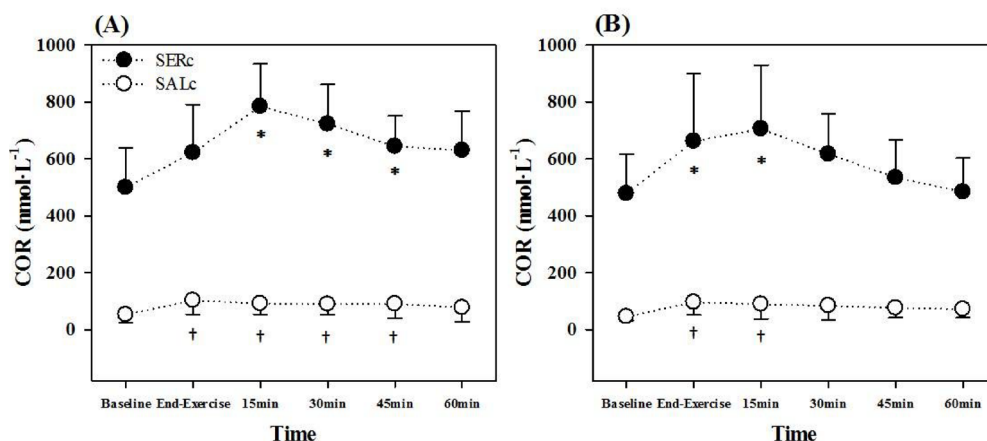


FIG. 2. Comparison of SERc and SALc responses during recovery from MEx (A) and PEx (B). Symbols denote a significant difference compared to the baseline value in SERc (*) and SALc (†) ($p<0.05$).

SERc of a stress response as it more accurately measures the amount of unbound cortisol compared to SERc [4, 10]. SERc and SALc were consistently higher with MEx than with PEx, as expected given that exercise intensity ($\geq 60\%$ VO_{2Max}) strongly correlates with COR [1].

SALc levels were comparable with MEx and PEx (Table 1), but SALc responses during recovery from MEx differed from those of SERc in that SALc levels declined initially and plateaued at 15 – 45 min of recovery, and remained above baseline at 60min of recovery. Similarly, SALc levels declined following PEx by 5 – 9% for each 15 min of recovery, but levels also remained above baseline at 60min of recovery. This indicates that, although peak SALc doubled following MEx and PEx, cessation of exercise either did not result in continued elevated expression of COR (as compared with SERc following MEx and PEx) or metabolism was altered. Possible reasons are that the salivary glands are one of the sites of activity of 11β -hydroxysteroid dehydrogenase type 2 (11β -HSD2) enzymes [11] involved in conversion of cortisol to inactive cortisone and may have functioned at an enhanced degree with elevated SALc levels after PEx. In contrast, the plateau effect on SALc noted at 15 – 45 min of recovery from MEx, suggests a slowing of SALc clearance that could be related to the fact that intense exercise does not result in increased 11β -HSD2 that may be related to a reduction in blood flow to the kidney (major site of 11β -HSD2 enzyme activity) due to redistribution to skeletal muscle [12].

Some differences noted in SERc and SALc metabolism during recovery may be important to consider when determining sampling times. Peak SERc levels occurred at 15 min of recovery, whereas SALc levels did not rise at any point in recovery. Therefore, when sampling for peak SERc levels following exhaustive exercises, it may be necessary to sample beyond the end-exercise point during recovery as shown 15 min post-exercise in the present study, whereas sampling of peak SALc levels should occur immediately at the termination of exercise. Further, at 1 hr of recovery from MEx, SERc was essentially equivalent to end-exercise levels, while PEx related SERc neared baseline levels. This may indicate that higher levels of SERc, during recovery from the intensive activity of MEx, led to saturation of 11β -HSD2 and slowed SERc clearance. Therefore, when sampling for SERc at 60min post-recovery from PEx, it should be anticipated that levels will have essentially returned to baseline values and, following MEx, will be nearly equivalent to those at end exercise. This

indicates that sampling of SERc post-MEx, to determine clearance, may have to be carried out over a longer period of time than 60min in order to ascertain when levels actually return to baseline. Similarly, because SALc levels after both MEx and PEx remained elevated above baseline at 60min of recovery (yet below end-exercise, peak levels), sampling to determine clearance returning to baseline values would require a longer sampling period.

Study limitations include that the present results were based on a robust group of male subjects that limits the implication of the results to other populations. Also, COR sampling was carried out through only 60 min of recovery and thus further insight into the clearance tendency of SERc and SALc for a longer period of recovery is limited.

CONCLUSIONS

The timing of sampling for peak COR levels responding to exhaustive exercise must take into account the source of biological samples analyzed as well as the type of exercises performed. In the present study, despite high correlation and agreement between SERc and SALc levels, peak SERc levels were attained at 15min of recovery, whereas peak SALc levels were attained immediately upon exercise cessation in both MEx and PEx conditions. Furthermore, clearance of SERc and SALc was slower following MEx than PEx while SALc were still below the peak, end-exercise levels at the end of 60min recovery. Taken in the aggregate, SALc is a sensitive, reliable method to determine COR responses following exercise in addition to its non-invasive, easy-sampling advantages.

Acknowledgements

The authors thank the subjects for their enthusiastic participation in the study. The present study was performed from internal operating funds of the National Personal Protective Technology Laboratory.

Conflict of Interest: The authors identify no conflicts of interest in the conduct of the present study. Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the views of National Institute for Occupational Safety and Health. Mention of commercial products does not constitute endorsement by National Institute for Occupational Safety and Health.

REFERENCES

- Gatti R, De Palo EF. An update: salivary hormones and physical exercise. *Scand J Med Sci Sports*. 2011;21(2):157-169.
- Hansen AM, Garde AH, Persson R. Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: a review. *Scand J Clin Lab Invest*. 2008;68(6):448-458.
- Raff H, Findling JW. A physiologic approach to diagnosis of the Cushing syndrome. *Ann Intern Med*. 2003;138(12):980-991.
- McGuigan MR, Egan AD, Foster C. Salivary cortisol responses and perceived exertion during high intensity and low intensity bouts of resistance exercise. *J Sports Sci Med*. 2003;3(1):8-15.
- Bruce RA, Kusumi F, Hosmer D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am Heart J*. 1973;85(4):546-562.
- Del Corral P, Mahon AD, Duncan GE, Howe CA, Craig BW. The effect of exercise on serum and salivary cortisol in male children. *Med Sci Sports Exerc*. 1994;26(11):1297-1301.
- Neary JP, Malbon L, McKenzie DC. Relationship between serum, saliva and urinary cortisol and its implication during recovery from training. *J Sci Med Sport*. 2002;5(2):108-114.
- Vining RF, McGinley RA, Maksytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum

- cortisol. *Ann Clin Biochem.* 1983;20(Pt 6):329-335.
9. McNaughton L, Bentley DJ, Koepfel P. The effects of a nucleotide supplement on salivary IgA and cortisol after moderate endurance exercise. *J Sports Med Phys Fitness.* 2006;46(1):84-89.
10. Stupnicki R, Obminski Z. Glucocorticoid response to exercise as measured by serum and salivary cortisol. *Eur J Appl Physiol Occup Physiol.* 1992;65(6):546-549.
11. Walker BR, Seckl JR. Cortisol Metabolism. In: Bjorntorp P editor. *International Textbook of Obesity.* New Jersey: John Wiley & Sons Ltd; 2001. p. 245.
12. Dovio A, Roveda E, Sciolla C, Montaruli A, Raffaelli A, Saba A, Calogiuri G, De Francia S, Borrione P, Salvadori P, Carandente F, Angeli A. Intense physical exercise increases systemic 11 β -hydroxysteroid dehydrogenase type 1 activity in healthy adult subjects. *Eur J Appl Physiol.* 2010;108(4):681-687.