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SHORT COMMUNICATION

Correlation between plasma and saliva adrenocortical hormones in response to submaximal exercise

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Abstract This study examined the relationships between plasma and saliva adrenocortical hormones in response to long-duration submaximal exercise. In nine healthy, physically active, female volunteers, blood and saliva samples were taken at rest and every 30 min during a 120-min cycling trial at 50–55% VO_{2max} for cortisol and dehydroepiandrosterone (DHEA) analysis. Correlation analysis revealed a moderate but significant relationship between plasma and saliva cortisol ($r = 0.35$, $P < 0.02$) and plasma and saliva DHEA ($r = 0.47$, $P < 0.001$) during the submaximal exercise. When expressed in percent of resting values, the correlations between the plasma and saliva concentrations were higher for both hormones during the exercise (cortisol: $r = 0.72$; DHEA: $r = 0.68$, $P < 0.001$). The results thus suggest that, even under prolonged exercise conditions, non-invasive saliva samples may offer a practical approach to assessing pituitary–adrenal function, especially when compared with individual basal values.

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Introduction

Saliva provides a convenient non-invasive way to determine adrenocortical [i.e., cortisol and dehydroepiandrosterone (DHEA)] hormone concentrations for the assessment of hypothalamic-adrenal–pituitary axis (HPA) activity in patients with adrenal insufficiency or posttraumatic stress disorder and in healthy subjects [1–7]. Given the positive correlations between blood and saliva values at rest, the influence of physical exercise on HPA has been determined using saliva cortisol and DHEA measurements in a number of studies [8–11]. However, few studies [12–16] have sought to determine whether the relationship between blood and saliva cortisol concentrations is maintained during exercise, and only one [12] tested the correlation between blood and saliva DHEA in response to exercise. Moreover, to our knowledge, no study has focused on long-duration exercise.

This study therefore evaluated the relationships between blood and saliva cortisol and DHEA concentrations during a 120-min cycling trial at 50–55% VO_{2max} in healthy, physically active women.

Materials and methods

Nine healthy, physically active, female volunteers (age: 20.3 ± 0.4 years; weight: 58.7 ± 1.9 kg) agreed to participate in the study after being informed of the nature of the experiments. Ethical committee approval and written informed consent were obtained. The women had been

cycling and/or running two–three times per week for at least 3 years and were screened with a medical history and physical examination. They were required to have been taking a low-dose oral contraceptive (OC) pill continuously over the past 12 months. They were also asked to abstain from intensive exercise and any caffeine and alcohol for 24 h before each trial, which was always conducted during the second part of the menstrual cycle.

In the month before the experiment, an incremental test for maximum oxygen uptake (VO_{2max}) was conducted following standard laboratory procedure on a Monark cycle ergometer (model 918E; Monark-Crescent, Varberg, Sweden) in order to select a power output eliciting 50–55% of VO_{2max}. Mean VO_{2max} was 41.1 ± 1.6 ml min^{-1} kg^{-1}. Trials were held at the same time of day (1000–1100 hours) for each subject in order to prevent diurnal variations in hormonal responses. Subjects were asked to come to the laboratory at 0900–1000 hours, 1 h after ingesting a small meal, which was identical for each trial. Dietary consistency (about 500 kcal) was confirmed through self-reported diet records and questioning before each trial. After insertion of a catheter into a superficial forearm vein (0930–1030 hours), subjects then rested (30 min) and, between 1000 and 1100 hours, after resting blood and saliva samples were taken, they exercised at 50–55% VO_{2max} for 2 h. Blood and saliva samples were taken every 30 min during exercise and water was given ad libitum during exercise.

One milliliter of unstimulated saliva was collected immediately after blood collection, using Salitubes (DRG Diagnostic, Germany). The Salitubes were promptly stored within the hour at −20°C until analysis. Each sample had to be frozen, thawed, and centrifuged at least once to separate the mucins. Blood samples (3 ml) were placed in a chilled tube containing EDTA, promptly centrifuged at 3,000*g* for 10 min at 4°C, and stored at −72°C until assays. Enzyme-linked immunosorbent assays (ELISA) were used for the plasma and saliva analyses: DHEA and cortisol (kits from DRG Diagnostic: DHEA (plasma): EIA-3415; DHEA (saliva): SLV-3012; cortisol (plasma): EIA-1887; cortisol (saliva): SLV-4635). Assays were made in duplicate and coefficients of variation for all parameters were always <10%.

Data are presented as mean values ± standard error of the mean (SE). Differences in the measured hormonal variables were statistically analyzed for time using an ANOVA. Correlations between plasma and saliva values were calculated using Pearson's product-moment correlation test. The null hypothesis was rejected at $P < 0.05$.

Results

No significant change in either basal plasma or saliva cortisol and DHEA concentrations was observed during the trial, although a tendency toward an increase in plasma DHEA was noted ($P = 0.064$) (Fig. 1).

The plasma and saliva cortisol concentrations were significantly correlated during submaximal exercise ($r = 0.35$, $P < 0.02$). When expressed in percent of resting

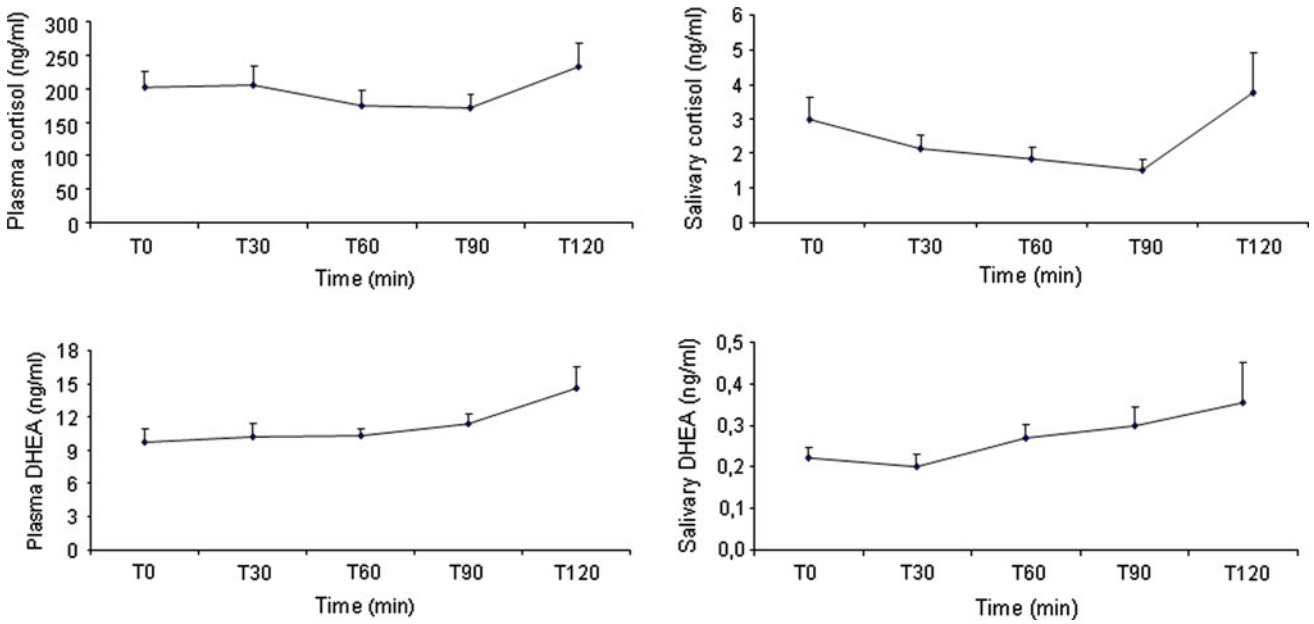

Fig. 1 Plasma and saliva (ng/ml) cortisol and dehydroepiandrosterone (DHEA) concentrations (mean ± SE) during a 120-min submaximal exercise at 50–55% VO_{2max}

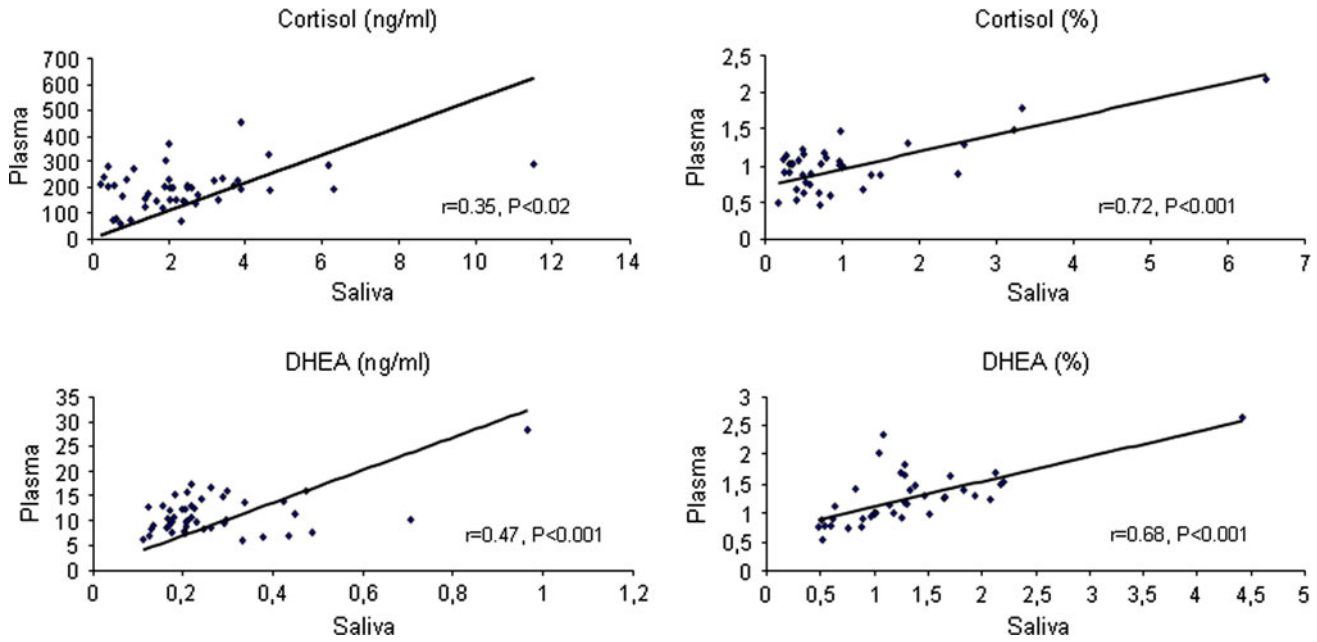

Fig. 2 Relationship between plasma and saliva cortisol and dehydroepiandrosterone (DHEA) during a 120-min submaximal exercise at 50–55% VO_{2max} expressed in concentrations (ng/ml) and in percent of resting values

values, this correlation appeared to be higher ($r = 0.72$, $P < 0.001$) (Fig. 2). Plasma DHEA was significantly correlated with saliva DHEA during the submaximal exercise ($r = 0.47$, $P < 0.001$). Similar to cortisol, plasma and saliva DHEA showed a stronger correlation ($r = 0.68$, $P < 0.001$) when expressed in percent of resting values (Fig. 2).

Discussion

The findings of the present study point to a moderate but significant relationship between plasma and saliva cortisol and DHEA concentrations during submaximal exercise. When expressed in percent of resting values, these correlations appeared to be much higher.

We found no significant change in the basal cortisol and DHEA concentrations in either plasma or saliva in response to submaximal exercise, although plasma DHEA concentration tended to increase. The literature reports conflicting results, but overall most studies have suggested that both exercise intensity and duration play an important role in exercise concentrations. A transient decrease in blood cortisol content during low workloads was described in an earlier publication [16], whereas a rapid change in cortisol concentration coincided in most cases with the onset of lactic acid accumulation [16, 17]. However, other studies did not report an increase in blood cortisol after exercise at workloads over 70% of VO_{2max}, and these results can be interpreted as an unusually rapid rate of cortisol removal [18]. Tremblay et al. [19] demonstrated that exercise duration also plays a significant role in exercise cortisol concentrations. The authors tested the plasma cortisol and DHEA sulfate responses in endurance-trained males during three treadmill runs of 40, 80, and 120 min at 55% of VO_{2max}. Plasma cortisol only increased in response to the 120-min run, whereas DHEA sulfate increased in a dose–response manner, with the greatest increases observed during the 120-min run. To our knowledge, only one study [20] compared the effects of several exercise intensities (44.5 ± 5.5%, 62.3 ± 3.8%, and 76.0 ± 6.0% of VO_{2max}) on saliva cortisol response in healthy, recreationally active subjects during a 1-h submaximal trial. Saliva DHEA was not investigated, but the authors reported that saliva cortisol was significantly increased only at 76% VO_{2max} at the end of exercise. They concluded that only high intensity and long duration resulted in significant elevations of saliva cortisol. In view of the results obtained in the present study, it can therefore be hypothesized that a longer duration at this relatively low intensity would have been necessary to stimulate a significant increase in plasma and saliva levels of cortisol and DHEA in our physically active subjects. Moreover, the decrease in cortisol and DHEA over the course of the day due to the nycthemeral rhythm may have counteracted a small increase due to exercise.

Although many studies have reported a significant correlation between plasma and saliva cortisol concentrations at rest, few exercise data have been published, especially regarding long-duration exercise. Del Coral et al. [13] and

O'Connor and Corrigan [14] observed a significant relationship between serum and saliva cortisol during a 30-min exercise at 70% of VO_{2max} in both adults and children, with respective correlations of $r = 0.60$–0.90 ($P < 0.01$) and $r = 0.46$–0.90 ($P < 0.05$). Port [16] conducted incremental exercise tests (4 min at each workload with 50-W increments) and reported a high correlation ($r = 0.86$, $P < 0.001$) for submaximal work, but not at maximal effort, suggesting that exercise intensity may influence these correlations. Moreover, the high variability in cortisol responses to exercise likely complicates the connection between serum and saliva. In a study with an isokinetic protocol, Paccotti et al. [15] evaluated serum and saliva cortisol responses in physically trained and untrained participants. They observed no significant correlation between the two values. The relationship between serum and saliva was markedly non-linear, but after logarithmic transformation of the raw data, a significant positive correlation was apparent ($r = 0.62$, $P < 0.001$). Lastly, Cadore et al. [12] showed a significant relationship between serum and saliva cortisol before ($r = 0.52$, $P = 0.05$) and after ($r = 0.62$, $P = 0.001$) a session of resistance exercise (75% of 1 RM) in healthy men. His team was the only one to also investigate exercise serum and saliva DHEA concentrations, and they noted a high correlation before ($r = 0.68$, $P < 0.001$) and after ($r = 0.70$, $P < 0.001$) the resistance exercise. To our knowledge, no study has yet investigated the relationship between blood and saliva DHEA during submaximal exercise. In agreement with the previously mentioned studies, we obtained a significant but more moderate correlation between plasma and saliva samples for both cortisol ($r = 0.35$, $P < 0.02$) and DHEA ($r = 0.47$, $P < 0.001$), which can be explained by our exercise protocol. However, when expressed in percent of resting values, the correlations between these two parameters during exercise appeared much higher, i.e., $r = 0.72$ ($P < 0.001$) for cortisol and $r = 0.68$ ($P < 0.001$) for DHEA. In order to take account of the large inter-individual differences, each subject should serve as his or her own control to assess pituitary–adrenal stimulation during submaximal exercise.

In summary, we found that the plasma and saliva values of cortisol and DHEA were correlated in physically active women. It thus appears that saliva adrenocortical concentrations can be used as a reference for their respective blood concentrations in response to submaximal exercise, especially when compared with individual basal values.

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Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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