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**SHORT COMMUNICATION**

# The diurnal patterns of cortisol and dehydroepiandrosterone in relation to intense aerobic exercise in recreationally trained soccer players

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**Abstract**

Diurnal patterns of cortisol and dehydroepiandrosterone (DHEA) secretion, the two main peripheral secretory products of the hypothalamic–pituitary–adrenal neuroendocrine stress axis, have been well characterized in rest conditions but not in relation to physical exercise. The purpose of this investigation was therefore to determine the effects of an intense 90-min aerobic exercise on the waking diurnal cortisol and DHEA cycles on three separate days [without exercise, with morning exercise (10:00–11:30 h), and with afternoon exercise (14:00–15:30 h)] in nine recreationally trained soccer players. Saliva samples were collected at awakening, 30 min after awakening, and then every 2 h from 08:00 to 22:00 h. A burst of secretory activity was found for cortisol ($p < 0.01$) but not for DHEA after awakening. Overall, diurnal decline for both adrenal steroids was observed on resting and exercise days under all conditions. However, there was a significant increase in salivary cortisol concentrations on the morning-exercise and afternoon-exercise days at, respectively, 12:00 h ($p < 0.05$) and 16:00 h ($p < 0.01$), versus the other trials. This acute response to exercise was not evident for DHEA. The results of this investigation indicate that 90 min of intense aerobic exercise does not affect the circadian pattern of salivary adrenal steroids in recreationally trained athletes over a 16-h waking period, despite a transitory increase in post-exercise cortisol concentration. Further studies are necessary to determine whether these results are applicable to elite athletes or patients with cortisol or DHEA deficiency.

**Keywords:** *Adrenal steroids, afternoon exercise, circadian rhythm, morning exercise, physical stress, saliva*

## Introduction

The role of adrenal steroids [i.e. cortisol and dehydroepiandrosterone (DHEA)] during exercise and stress situations has attracted substantial attention for three decades. As steroid hormone concentrations can be measured noninvasively in saliva, it is now possible to follow these hormone responses on a longitudinal basis without imposing additional stress. Indeed, the correlation between saliva and serum concentrations is high in healthy and pathological subjects both at rest and during exercise (Port 1991; Paccotti et al. 2005; Wirtz et al. 2007; Cadore et al. 2009; Baillot et al. 2011).

Cortisol and DHEA are steroid hormones that are secreted in a circadian fashion (Passelergue et al. 1995; Hucklebridge et al. 2005) with the highest concentrations observed in the morning and the lowest in the evening. Thus, normal saliva values for cortisol decrease by more than 80% during the day, from a mean of 12–15 nmol/l at 08:00 h to a mean of 4–5 nmol/l in the early afternoon and 2 nmol/l in the late afternoon (Passelergue et al. 1995; Hucklebridge et al. 2005). For DHEA, the normal saliva values in men and women subjects, ranging from 18 to 46 years of age, decrease from a mean of 1.4 nmol/l in the morning to 0.7 nmol/l 12 h post-awakening, with no significant decrease between 3 and 12 h post-awakening (Hucklebridge et al. 2005). The main distinction between cortisol and DHEA secretion is that, although DHEA concentration is elevated in post-awakening samples compared with those later in the day, there is no evidence of an awakening stimulatory burst of DHEA secretory activity (Hucklebridge et al. 2005).

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Numerous studies have examined saliva cortisol responses to exercise (O'Connor and Corrigan 1987; Passelergue et al. 1995; Passelergue and Lac 1999; Le Panse et al. 2010), and it appears that a relatively high intensity of exercise is needed (>60% $VO_2$ max) to induce an acute increase in salivary cortisol concentration versus rest values. Few studies have investigated DHEA responses to exercise (Cadore et al. 2009; Le Panse et al. 2010) but they seem to show comparable results, with a significant increase in DHEA secretion seen during intense exercise. Only two studies (Minetto et al. 2008; Georgopoulos et al. 2011), however, have examined the effects of physical exercise on the circadian rhythm of saliva cortisol, and only with a few isolated saliva sample collections, either after 7 days of intensive training (Minetto et al. 2008) or shortly after an artistic gymnastics competition (Georgopoulos et al. 2011). To our knowledge, no study to date has examined the daily variations in saliva DHEA with physical exercise.

As greater emphasis is being placed currently on physical activity, in terms of health status maintenance or athletic performance, it appears important to determine whether acute and chronic physical exercise alters the patterns of steroid secretion in both healthy and pathological subjects. In the first step, the objective of this investigation was therefore to determine the effects of an acute, heavy aerobic exercise on the waking diurnal pattern of salivary cortisol and DHEA concentrations in healthy, recreationally trained, young male soccer players. We hypothesized that a single bout of intense exercise, independent of time of day, would not affect the cortisol and DHEA patterns and that the two adrenal steroids would show the same secretory response to exercise.

## Methods

### *Subjects*

All procedures were approved by the local Ethics Committee and were carried out in accordance with the Declaration of Helsinki.

The group consisted of nine healthy males, all recreationally trained soccer players (mean ± SEM: body weight 78.4 ± 3.3 kg; age 19.9 ± 0.4 years) recruited on the basis of verbal and written information about the study. The subjects trained an average of 60–90 min, 2–3 times per week, at an intensity close to that of the test, and all agreed to participate in the study with written consent after being informed of the nature of the experiments.

### *Exercise*

Exercise intensity was individually based on maximal heart rate, previously determined for each subject. The exercise session consisted of 45 min of running at 70% of peak heart rate followed by interval training of 2 × 15 min at 80% peak heart rate, with a 5-min recovery between bouts and 5 × 1 min at maximal heart rate, with a 1-min recovery between bouts. Electronic pulse monitors (S710 polar®) were used to control exercise intensities.

Each subject completed the same exercise session twice: 1 day in the morning (10:00–11:30 h) and 1 day in the afternoon (14:00–15:30 h).

### *Experimental timeline*

The rest day and the two exercise days were randomized and separated by 48–72 h.

For each of the three experimental days (rest day, morning-exercise day, afternoon-exercise day), the first saliva sample was collected from each subject at awakening (between 06:00 and 06:30 h) and 30 min after awakening in a fasting state. Subjects then took their breakfast at least 30 min before the next sampling. Samples were then collected every 2 h from 08:00 to 22:00 h. Compliance with the saliva sampling protocol was monitored and confirmed by a written questionnaire collected by an investigator at the end of each experimental day. Except for the completion of the exercise protocol, the subjects kept to their normal daily routines, that is, they maintained similar exercise patterns and normal food intake throughout the duration of the experiments and abstained from intense exercise and any caffeine and alcohol for 24 h before each trial.

They were required to avoid eating, drinking, chewing gum, or brushing teeth for 30 min before saliva sampling.

### *Saliva collection and analysis*

Unstimulated saliva was collected by the subjects themselves using Salitubes (DRG Diagnostics, Marburg, Germany). No sample was collected from an athlete with oral disease, inflammation, or lesion. Salitubes were promptly stored at 4°C for an hour and at −20°C for 3 days until analysis. Each sample was frozen, thawed, and centrifuged at least once to separate mucins. Enzyme-linked immunosorbent assays were used for the saliva cortisol and DHEA analyses (DRG Diagnostics). Assays were made in duplicate, and coefficients of variation for all parameters were always <10%. The analytical sensitivity for cortisol and DHEA was 0.1 nmol/l and 15.4 pmol/l, respectively. All samples were analyzed according to a blind procedure and were decoded only after analyses were completed.

### *Statistical analysis*

Data are presented as mean values ± SEM.

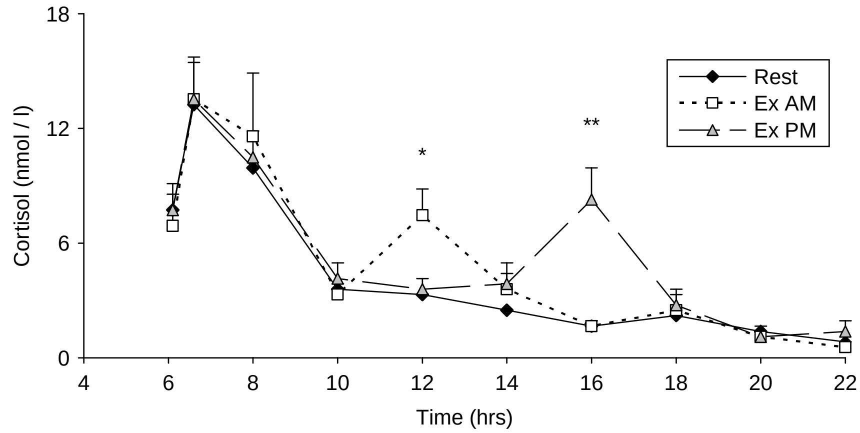


Figure 1. Diurnal pattern of salivary cortisol concentration on the rest day (*rest*) and the two exercise days [(morning-exercise day (*ex AM: 10:00–11:30 h*); afternoon-exercise day (*ex PM: 14:00–15:30 h*)] (mean ± SEM) in nine recreationally trained soccer players (two-way ANOVA). Concentrations were higher in the early morning than in the evening ($p < 0.01$, ANOVA) and highest 30 min after awakening ($p < 0.01$, Tukey), irrespective of exercise. *$p < 0.05$, *ex AM versus rest and ex PM*; **$p < 0.01$, *ex PM versus rest and ex AM*.

A two-way ANOVA was conducted with the following two factors: time sampling (10 levels: awakening, 30 min post-awakening, 08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 22:00 h) and sampling day (three levels). A Tukey post-hoc test was used to determine differences when a significant F ratio was observed. Correlations between hormonal saliva values were calculated using Pearson's product-moment correlation test. The null hypothesis was rejected at $p \leq 0.05$.

## Results

The results of the saliva sampling for cortisol and DHEA on the rest day and the two exercise days are shown in Figures 1 and 2, respectively. Under each experimental condition (rest/morning-exercise/afternoon-exercise days), we observed a classic circadian rhythm for these steroids (cortisol: $F_{9,260} = 30.2$, $p < 0.01$; DHEA: $F_{9,260} = 4.74$, $p < 0.01$) with a higher concentration in the morning and a lower concentration in the evening hours, and a significant ($p < 0.01$) burst of concentration 30 min after awakening for cortisol but not for DHEA. A significant decrease in cortisol concentration was observed at each time point compared with awakening, except for the 30-min post-awakening and 08:00 samples. DHEA fell from high awakening values to significantly lower values at 12:00 h ($p < 0.05$), with no evidence of significant change thereafter.

No main effect for day of sampling was evident, and the diurnal patterns of both cortisol ($F_{2,267} = 1.82$, $p > 0.05$) and DHEA ($F_{2,267} = 0.71$, $p > 0.05$) concentrations were not different between the three conditions. However, when comparing matched time points, a significant increase was observed for cortisol

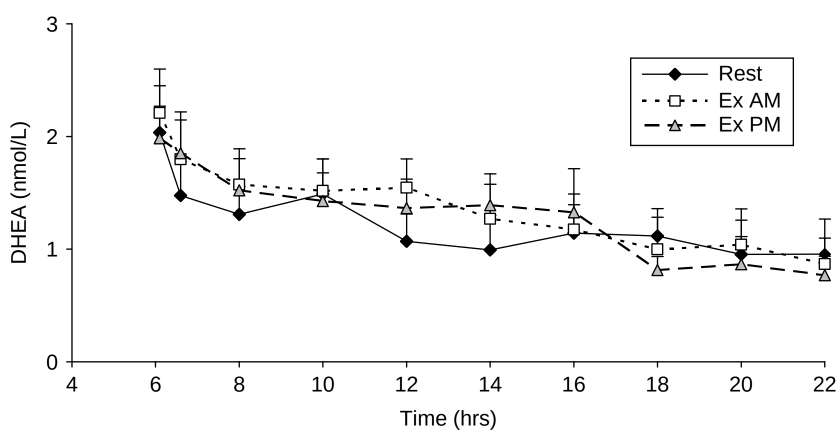


Figure 2. Diurnal pattern of salivary DHEA on the rest day (*rest*) and the two exercise days [(morning-exercise day (*ex AM: 10:00–1:130 h*); afternoon-exercise day (*ex PM: 14:00–15:30 h*)] (mean ± SEM) in nine recreationally trained soccer players (two-way ANOVA). No significant group differences. Concentrations were higher in the early morning than in the evening ($p < 0.01$, ANOVA), irrespective of exercise.

at 12:00 h ($p < 0.05$) on the morning-exercise day versus the rest day and the afternoon-exercise day. Similarly, cortisol concentration at 16:00 h was higher ($p < 0.01$) on the afternoon-exercise day compared with the rest day and the morning-exercise day.

A relatively weak but significant ($r = 0.23$, $p < 0.05$) correlation was found between the concentrations of cortisol and DHEA whatever the time of sampling.

## Discussion

This study contributes to the limited literature examining the impact of physical exercise on diurnal steroid secretory patterns. The major result is that the physical stress generated by 90 min of intense aerobic training induced a transitory increase in saliva cortisol but not DHEA post-exercise, without affecting the circadian pattern of these adrenal steroids in recreationally trained athletes over a 16-h waking period.

The finding that the secretory activities of cortisol and DHEA showed differences in patterns in this group of healthy, recreationally trained soccer players reinforces the evidence for a dissociation in their secretory activity, despite their common secretagogue adrenocorticotropic hormone (ACTH; Oskis et al. 2012). First, whatever the sampling day, we observed an awakening stimulatory burst of cortisol secretory activity but not of DHEA. This finding is in line with previous studies on both female and male adults and adolescents (Hucklebridge et al. 2005; Oskis et al. 2009, 2012). Another signal, in addition to pituitary ACTH, is known to be implicated in regulating the post-awakening cortisol secretory episode (Hucklebridge et al. 2005). It is possible that the second signal emanates from the hypothalamic suprachiasmatic nucleus, as light exposure has been demonstrated to amplify the awakening cortisol response (Hucklebridge et al. 2005). In parallel, and in agreement with the literature, the diurnal pattern of cortisol appeared more marked than that of DHEA, with a decrease by more than 80% for cortisol and only about 50% for DHEA over this 16-h waking period. It should also be noted that our DHEA values were slightly higher than in the previous studies, with a possible explanation being the age or the recreationally trained status of our subjects.

Cortisol and DHEA responses to acute high-intensity exercise may present different dynamics in relation to the exercise modality. Passelergue and Lac (1999) found a rapid return of cortisol concentrations to pre-exercise values within 1 h after the end of a weight-lifting competition, while Paccotti et al. (2005) and Minetto et al. (2006) found cortisol and DHEA levels still elevated 2 h after the end of an isokinetic fatiguing task. In the present study, saliva samples were collected 30 min after the end of intense aerobic exercise on the two exercise days and no significant differences were observed in either cortisol or DHEA concentrations between the resting and exercise conditions, except for significant increases in cortisol concentration at 12:00 on the morning-exercise day ($p < 0.05$) and at 16:00 on the afternoon-exercise day ($p < 0.01$). It is interesting to note that the same exercise did not result in an increase in DHEA concentration. This lack of change in post-exercise DHEA secretion may be due to the time of collection, with a return to basal values in the post-exercise sample, possibly linked to the shorter elimination half-life of DHEA compared with cortisol. However, the dissociation in the secretory response of the two steroids to exercise, with the greater post-exercise increase in cortisol reflecting sympathetic innervation to the zona fasciculata, cannot be ruled out.

The lack of effectiveness of this intense aerobic physical stress on diurnal cortisol patterns is nevertheless in contradiction with previous investigations of only cortisol patterns. Minetto et al. (2008) collected samples in the morning for the analysis of the awakening cortisol response and at midnight (four samples per day). They found a greater increase in both awakening and midnight salivary cortisol responses after 7 days of intensive training. In another study, with saliva samples collected in the morning before training and in the afternoon shortly after an artistic gymnastics competition (two samples per day) (Georgopoulos et al. 2011), no difference was found between morning and afternoon salivary cortisol levels in either male or female elite gymnasts. The authors concluded that the diurnal rhythm of salivary cortisol had been abolished due to strenuous training and competition conditions. It must, however, be noted that only a few isolated samples were collected in the two aforementioned studies. Moreover, the lack of change between morning and afternoon cortisol values in the elite gymnasts seems to reflect only the cortisol increase induced by competition just before the afternoon collection (Le Panse et al. 2010) and not by an abolished circadian rhythm.

In conclusion, our study rules out any significant repercussion of a single heavy aerobic exercise in the morning or afternoon on the diurnal cortisol and DHEA patterns in recreationally trained subjects, despite a transitory post-exercise cortisol increase. This work provides an important baseline for patterns of saliva steroid secretion in healthy recreationally trained athletes in relation to intense aerobic exercise. Further studies are necessary to determine whether these results are applicable to (a) elite athletes who are known to be subjected to much greater workloads and (b) pathological subjects with cortisol or DHEA deficiency (Wirtz et al. 2007; Baillot et al. 2012).

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