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Diurnal patterns of salivary alpha-amylase and cortisol secretion in female adolescent tennis players after 16 weeks of training

Edith Filaire [a,*], Jose Pedro Ferreira [b], Miguel Oliveira [b], Alain Massart [b]

[a] *Laboratoire CIAMS, EA4532, Université Paris-Sud, Université Orléans, UFRSTAPS, 2 allée du Château, 45067 Orléans Cedex, France*
[b] *Centro de Investigação do Desporto e da Activida de Física, Universidade de Coimbra, Faculdade de Ciências do Desporto e Educação Física, Estádio Universitário de Coimbra, 3040-156 Coimbra, Portugal*

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Summary We examined the effects of 16 weeks of training on diurnal pattern of salivary alpha-amylase (sAA), cortisol, and the ratio of sAA over cortisol (AOC) in 12 national adolescent female tennis players. Stress and recovery were also evaluated using the Recovery-Stress-Questionnaire for Athletes-RESTQ-Sport. Data were collected after a 2-week rest (January, W_0), and 4 months after W_0 (W_{16}). Subjects collected five saliva samples throughout a day. While all participants displayed the previously shown decrease after awakening in adolescents at W_0, they showed a rise in the alpha-amylase awakening response and a higher alpha-amylase activity output ($p < 0.01$) at W_{16} compared to W_0. For the daily rhythm of cortisol we found subjects having a low overall output of salivary cortisol ($p < 0.01$) and a blunted response to awakening at W_{16}. Furthermore, an increase in the ratio AOC at W_{16}, and a negative correlation between this ratio and Sport-specific recovery score. Our findings offer support for the hypothesis that increase of training load during the study period induced asymmetry activation between the two stress systems, in relation to psychological alterations and performance decrease. These results provide encouragement to continue exploring the impact of training program using a psychobiological approach among young athletes in order to prevent fatigue and preserve the health of these athletes.

1. Introduction

Young athletes, wishing to reach the highest level of performance, engage in intensive training program that requires several hours of training per day (Georgopoulos et al., 2011). This pressured schedule often induces little opportunity for recovery (Matos et al., 2011). A sustained mismatch between the load of training and recovery can lead to overreaching (OR). OR is characterized by a short-term decrement in sport specific performance and is often coupled with psychological symptoms (Brink et al., 2012). OR can be further classified as either functional overreaching (FOR) or non-functional overreaching (NFOR) with the criteria for each based on the duration of performance decrement and severity of symptoms (Meeusen et al., 2006; Matos et al., 2011). FOR refers to

* Corresponding author. Tel.: +33 238 417 178.
E-mail address: edith.filaire@univ-orleans.fr (E. Filaire).

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a short-term performance decrement (i.e., days to weeks) with a planned recovery period, while NFOR is the more severe condition where performance decrement lasts longer (weeks or months) and usually presents with more severe symptoms. The overtraining syndrome (OTS) is the final stage of the continuum with recovery periods lasting from months to years (Meeusen et al., 2006). The avoidance of OR and the achievement of optimal performance can be only realized when athletes are able to recover and optimally balance training stress and subsequent recovery (Kellmann and Kallus, 2001). These complex effects of stress and recovery may be evaluated through the Recovery-Stress Questionnaire for Athletes (RESTQ-Sport), which assesses the frequency of experienced stressors and regeneration related activities (Nederhof et al., 2008).

In comparison to adults, our knowledge of the OTS in elite young athletes is lacking, and knowledge regarding health outcomes related to youth sport specialization is limited (Capranica and Millard-Stafford, 2011). Some studies indicate an incidence of 30%, with relatively higher occurrence seen in individual sport, females and those competing at the highest representative levels (Raglin, 1993; Matos et al., 2011). Recently, the International Olympic Committee has recommended international federations to monitor the volume and intensity of training and competition, considering that young athletes could be exposed to an excessive psychophysiological stress (Mountjoy et al., 2008). The availability of noninvasive lightweight equipment (i.e., portable lactate analyzers, saliva collectors) and the cooperation with sport federations allowed a multidisciplinary approach to examine physiological and psychological contributors to stress in individual athletes (Capranica and Millard-Stafford, 2011), and to evaluate the balance in allostasis (meaning literally "maintaining stability or homeostasis"), defined in terms of ability to achieve stability through change (Minetto et al., 2008).

The most common allostatic responses involve the sympathetic adrenomedullary system (SAM) with the secretion of catecholamines and the hypothalamic-pituitary-adrenal (HPA) axis with the secretion of cortisol (Urhausen et al., 1998). In a general manner, allostatic load is a framework, which asserts that the cumulative burden of stress manifests as physiological dysregulation across multiple, interrelated systems involved in restoring allostasis and maintaining healthy functioning in the presence of internal or environmental demands (McEwen, 1998). This "load" is typically indicated by elevated (or reduced) levels across neuroendocrine markers of the sympathetic-adrenal-medullary (SAM; i.e., adrenaline and noradrenaline) and hypothalamic-pituitary-adrenal (HPA) axis (i.e., cortisol) (Gallo et al., 2011). It has also been shown that the degree of dysregulation associated with chronic stress could depend markedly on subjective distress, personality and coping resources, and the social context in which stress occurs (Chun et al., 2006). Acute intense exercise activates the HPA axis, while intense physical training, by acting as a chronic repeated stress, leads to a reduced HPA axis reactivity (Urhausen et al., 1998). Salivary cortisol sampling has been used as a measure for HPA axis activity for quite some time (Kirschbaum and Hellhammer, 1994). One method to analyze cortisol secretory activity is the assessment of saliva cortisol levels after awakening when hormone concentrations strongly increase until 30 min after awakening and decrease thereafter (Pruessner et al., 1997). The cortisol awakening response (CAR; the difference in cortisol from awakening to some period after awakening) has been introduced as a measure of cortisol reactivity and appears to be a distinct phenomenon superimposing the circadian rhythm of cortisol (Wilhelm et al., 2007). CAR also allows repeated assessment and has been shown to have a high intra-individual stability (Clow et al., 2004). As alterations of the CAR may reveal subtle changes in the HPA axis activity, this parameter has been investigated in several patient groups. In many disorders including chronic fatigue syndrome, posttraumatic stress disorder, or sleep disorder, a blunted CAR has been observed when comparing patients with healthy controls (Fries et al., 2009). To our knowledge, limited information is available concerning the CAR and diurnal variations of cortisol concentrations in athletes, Georgopoulos et al. (2011) showing an abolished diurnal rhythm of salivary cortisol in elite artistic gymnasts. A blunted diurnal rhythmicity of cortisol in young male dancers was also reported by Strahler et al. (2010a). These researchers also observed a markedly reduced HPA activity in male martial artists (Strahler et al., 2010b). However, the reason for the reduced basal HPA activity in trained athletes is still unclear. On the contrary, Rohleder et al. (2007) showed that competitive dancing produces substantial increases in cortisol compared to a control day. These increases were not due to the physical load of dancing and responses did not habituate across competitions and were mostly elevated under highly focused conditions of threat, supporting the notion of a social self-preservation system that is physiologically responsive to threats to the social self.

Direct measurements of salivary adrenaline and noradrenaline do not seem to reflect SAM activity (Schwab et al., 1992). The search for a similar non-invasive and easily obtainable marker of the SAM activity has raised salivary alpha-amylase (sAA) as a promising candidate (Filaire et al., 2010; Granger et al., 2007). Pharmacological study in the human also provides direct evidence for the sensitivity of sAA to changes in adrenergic activity and specifically as a marker in reaction to psychological stress (Van Stegeren et al., 2006). sAA is the most important and abundant protein in saliva, and is mostly synthesized by the parotid gland. AA is an enzyme that breaks down starch into maltose and is also important in host defenses, inhibiting the adherence and growth of certain bacteria. Its secretion is under strong neurohormonal control (released upon sympathetic stimulation). Nater et al. (2007) reported a diurnal rhythm of salivary alpha-amylase secretion in humans with a decrease immediately after awakening and a subsequent steadily rise toward the afternoon and the evening. The same diurnal profile was shown in children and adolescents (Wolf et al., 2008). Since the early work of Chatterton et al. (1996), several studies have also underscored the usefulness of variations in sAA in reflecting changes in autonomic activity in sports and exercise (Kivlighan and Granger, 2006; Nater and Rohleder, 2009; Bocanegra et al., 2012; Diaz et al., 2012). In studies using different kinds of physical stimulation, a sympathetic-like response was shown for sAA with rapid increases immediately after the stressor and rapidly declining values afterwards, either in morning or afternoon exercise sessions (Allgrove et al., 2008; Chiodo et al., 2011; Capranica et al., 2012).