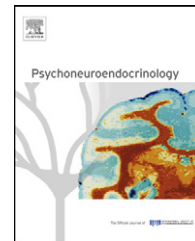




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# Effects of prolonged stress on salivary cortisol and dehydroepiandrosterone: A study of a two-week teaching practice

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**Summary** This study investigated variations in salivary levels of cortisol and dehydroepiandrosterone (DHEA) in a prolonged stressful situation (a two-week teaching practice). Thirty-three women for whom a two-week teaching practice at a kindergarten was scheduled were asked to collect saliva samples at awakening, 30 min after awakening, and bedtime at four time points: two weeks before the practice, the first week of the practice, the second week of the practice, and a few days after the practice. In addition, they completed questionnaires for assessing perceived stress and subjective moods on each day. A linear mixed model indicated that cortisol levels significantly increased during the first and second week of the practice compared with those before and after the practice period, and that DHEA levels significantly decreased after the practice period compared with those at the other time points. Further, cortisol awakening response after the practice period significantly reduced compared with that at the other time points. Scores of perceived stress and negative moods were also higher during the practice period. This study showed that prolonged stress affected cortisol and DHEA secretion during as well as after the stress period.

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## 1. Introduction

Psychosocial stress triggers a number of physiological changes such as activation of the sympathetic nervous system and hypothalamic–pituitary–adrenal (HPA) axis. Regarding these physiological changes, numerous studies have shown that acute psychological stress increases cortisol levels in blood and saliva (Dickerson and Kemeny, 2004). Moreover, cortisol is associated with health-related variables such as psychoses and cardiovascular disease; therefore, cortisol is a mediator between psychosocial stress and health (McEwen, 2000).

Many studies have also investigated the relationship between daily life stress and cortisol. For example, work stress (Steptoe et al., 2000), unemployment (Ockenfels et al., 1995), and divorce (Powell et al., 2002) were associated with increased morning or night-time cortisol levels. Recent studies also investigated the cortisol awakening response and the stress in daily life. Cortisol levels in plasma and saliva increased by 50–60% within 30 min after awakening. Recent studies have shown that the cortisol awakening response is a useful indicator of HPA activity (Clow et al., 2004). A meta-analysis by Chida and Steptoe (2009) showed that work-related stress and other types of life stress (e.g., financial strain, loneliness, and poor marital quality) were associated with an increased cortisol awakening response. It was also shown that the cortisol awakening response was diminished in patients with post-traumatic stress disorder and individuals with high levels of fatigue and burnout. Cortisol awakening response may provide valuable information about psychosocial factors and health.

However, most of the above studies cross-sectionally investigated the effects of daily life stress on cortisol levels. Further, some of the studies focused on academic stress to longitudinally investigate the effects of prolonged stress (lasting certain periods of time, e.g., a few weeks or months) on cortisol levels, and they showed that the cortisol levels increased in the evening (e.g., Murphy et al., 2010; Katsuura et al., 2010) and at awakening (Izawa et al., 2007; Weekes et al., 2008; Weik and Deinzer, 2010) during prolonged academic stress compared with the levels during non-stress periods; however, cortisol levels at various time points during prolonged stress situations were not reported. Examination schedules and preparation practices might have differed between students, and the starting point of the stressful period was unclear. Most of the studies also did not investigate cortisol secretion after the periods of academic stress.

The associations between psychosocial stress and dehydroepiandrosterone (DHEA) levels have also been recently investigated. DHEA is an androgen secreted by the zona reticularis of the adrenal cortex and is a precursor for sex steroids. The main secretagogue for DHEA is ACTH, and DHEA was reported to be secreted synchronously with cortisol during the night and day (Rosenfeld et al., 1971). DHEA may also affect certain brain functions by modulating neurotransmitter receptors such as GABA-A or NMDA receptors, and be involved in the pathophysiology of cognitive decline and mood disorders (Wolf and Kirschbaum, 1999). Acute psychosocial stress was reported to increase DHEA concentration (Shircliff et al., 2007; Izawa et al., 2008). However, to our knowledge, no studies have longitudinally investigated the effect of prolonged stress on DHEA levels. Further, DHEA has a balancing effect on cortisol levels. The effects of DHEA on

brain functions are believed to be opposite to those of cortisol. For example, it was reported that the cortisol/DHEA ratio was elevated in depressive patients (Young et al., 2002), and that a high cortisol/DHEA ratio in adolescents was predictive of persistent major depression (Goodyer et al., 2003). Moreover, under acutely stressful situations, the cortisol/DHEA ratio was reported to be correlated with a negative mood level (Izawa et al., 2008). However, the association between cortisol/DHEA ratio and mood under prolonged stress has not been investigated.

In this study, we used a two-week teaching practice as a prolonged stressful situation and evaluated undergraduate students for whom the practice was scheduled. In the Japanese education system, undergraduate students of the Department of Education have to undertake teaching practice at a kindergarten and elementary school for at least 10 days to obtain childcare- and education-related certificates (e.g., kindergarten teacher and elementary school teacher). The practices serve as good opportunities for the students to acquire skills, but they are highly stressful owing to unfamiliar environments and personal relationships, evaluation of the students' performances, and demanding tasks such as maintaining a practice diary (Saito et al., 2007). This study longitudinally investigated the effects of prolonged stress during a teaching practice on the secretion of cortisol and DHEA. This study focused on the following: first, we explored the effect of prolonged stress on endocrine activities (cortisol levels, DHEA levels, and cortisol/DHEA ratio) during the period of stress, with particular emphasis on diurnal patterns of cortisol and DHEA secretion (awakening and bedtime) and cortisol awakening response (changes from awakening to 30 min after awakening). Second, we explored the effect of prolonged stress after the period of stress because previous studies reported that prolonged stress had persistent effects after the period of stress (e.g. Deinzer et al., 2000). Third, we determined the associations between endocrine activities (cortisol levels, DHEA levels, and cortisol/DHEA ratio) and psychological distress in this situation because the above studies reported the associations between endocrine activities, particularly cortisol/DHEA ratio, and negative moods.

## 2. Methods

### 2.1. Participants

Thirty-three healthy female students for whom a two-week teaching practice was scheduled at a kindergarten participated in this study. The mean (standard deviation) of age and body mass index was 19.5 (3.3) years and 21.0 (2.7) kg/m<sup>2</sup>, respectively. All the participants were non-smokers and did not use medications (including oral contraceptives) or dietary supplements known to affect HPA axis activity. It was also confirmed that the participants had no history of psychiatric or physical diseases. Written informed consent was obtained before the commencement of the study, and the study was approved by the Ethical Committee of the university.

### 2.2. Measures

Saliva samples were used for DHEA and cortisol measurement. The participants were asked to expectorate saliva

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