



Diurnal patterns of salivary cortisol and DHEA using a novel collection device: Electronic monitoring confirms accurate recording of collection time using this device

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Summary The accurate indication of saliva collection time is important for defining the diurnal decline in salivary cortisol as well as characterizing the cortisol awakening response. We tested a convenient and novel collection device for collecting saliva on strips of filter paper in a specially constructed booklet for determination of both cortisol and DHEA. In the present study, 31 healthy adults (mean age 43.5 years) collected saliva samples four times a day on three consecutive days using filter paper collection devices (Saliva Procurement and Integrated Testing (SPIT) booklet) which were maintained during the collection period in a large plastic bottle with an electronic monitoring cap. Subjects were asked to collect saliva samples at awakening, 30 min after awakening, before lunch and 600 min after awakening. The time of awakening and the time of collection before lunch were allowed to vary by each subjects' schedule. A reliable relationship was observed between the time recorded by the subject directly on the booklet and the time recorded by electronic collection device ($n = 286$ observations; $r^2 = 0.98$). However, subjects did not consistently collect the saliva samples at the two specific times requested, 30 and 600 min after awakening. Both cortisol and DHEA revealed diurnal declines. In spite of variance in collection times at 30 min and 600 min after awakening, the slope of the diurnal decline in both salivary cortisol and DHEA was similar when we compared collection tolerances of ± 7.5 and ± 15 min for each steroid. These unique collection booklets proved to be a reliable method for recording collection times by subjects as well as for estimating diurnal salivary cortisol and DHEA patterns.

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1. Introduction

The hypothalamic pituitary adrenal axis (HPA) has been implicated in many homeostatic regulatory processes, most importantly the stress response (McEwen, 2003). Use of saliva for assessing steroid hormones has increased dramatically over the past two decades (Hellhammer et al., 2009; Laudenslager et al., 2005) and continues to increase exponentially. Increased use of saliva for assessing steroids is related to its relative ease of collection. Saliva samples can be easily collected in a laboratory setting for measuring the acute response to laboratory stressors (Dickerson and Kemeny, 2004) as well as in the home environment for epidemiological investigations which track the diurnal release (Adam and Kumari, 2009). However, these approaches are not without problems including protocol adherence which varies with specific instructions provided to the subjects, number of days of sample collection, as well as subject age (Halpern et al., 2012). The characteristics of the diurnal cortisol pattern may be affected by factors including early trauma (Gunnar and Vazquez, 2001) and/or psychopathology (Bao et al., 2004; Young et al., 2002).

The diurnal pattern of cortisol is further distinguished by a rapid rise in cortisol level that peaks approximately 30–40 min after awakening, known as the cortisol awakening response (CAR) (Chida and Steptoe, 2009; Clow et al., 2004; Hucklebridge et al., 2005) with a subsequent decline through the day to an early evening nadir (Oskis et al., 2009). The CAR and diurnal decline are thought to be under control by different CNS systems (Chida and Steptoe, 2009). The decline over the course of the day can be affected by acute and/or chronic stressors (Gunnar and Quevedo, 2007). The decline may be steep, flat and low, flat and high, as well as slowly rise after awakening (Miller et al., 2007). The diurnal pattern is influenced by adherence when specific collection times are fixed by study instructions (Broderick et al., 2004; Kudielka et al., 2003). The nature of the CAR is affected by a number of complex factors which can lead to increases or decreases in its magnitude (Chida and Steptoe, 2009). Disruption of the diurnal decline may have important predictive value with regard to multiple outcomes (Kumari et al., 2011; Spiegel, 2012). Salivary cortisol remains an important marker of stress activation in the individual (Hellhammer et al., 2009; Kudielka and Wust, 2010) but less is known with regard to diurnal salivary dehydroepiandrosterone or DHEA.

DHEA is often considered as counter-regulatory to cortisol (Kahl et al., 2006; Netherton et al., 2004) and reveals a similar diurnal pattern with DHEA in saliva highest in the morning with a nadir in late evening similar to cortisol. Unlike cortisol, salivary DHEA lacks an awakening rise (Hucklebridge et al., 2005) but similar to cortisol, DHEA is influenced by factors such as psychopathology (Goodyer et al., 2000; Ritsner et al., 2004; Shirtcliff et al., 2007). Lower morning levels of DHEA have been linked to depression as well as risk for affective disorders and schizophrenia (Gallagher et al., 2007; Goodyer et al., 2003; Kahl et al., 2006). In the context of the long term goals for a study of caregiver distress, depression, and intervention efficacy (Laudenslager et al., in preparation), the ability to characterize diurnal salivary cortisol as well as DHEA was fundamental to that project.

Notwithstanding the many important advances that have been made using home based saliva collection (Hellhammer et al., 2009), adherence to protocol remains problematic (Adam and Kumari, 2009; Broderick et al., 2004; Hellhammer et al., 2007; Nater et al., 2007). Herein, the term “adherence” as opposed to “compliance” refers to the extent to which a subject’s behavior coincides with study instructions (Lutfey and Wishner, 1999). When collecting saliva, the subject must adhere to study instructions including the time of collection, eating and drinking restrictions, transportation and handling of the sample such as refrigeration and/or freezing. The feasibility and reliability of salivary cortisol measured in the natural environment have been mixed (Adam and Kumari, 2009; Halpern et al., 2012). Typical collection packages include instructions, cotton roll devices for collecting and receptacles for the wetted cotton roll, a straw, or a vial for passive drool collection. A log book for recording time of collection is typically included with the collection device(s). The subject burden can be substantial adding to the likelihood of non-adherence. Various methods including color-coding, phone call reminders, face to face meeting, and electronic monitoring devices have been utilized to increase subject adherence.

Adherence with collection procedures is particularly crucial for accurate assessment of the CAR. The maximum CAR, occurring within a narrow window of 30–45 min after awakening, may be specifically sensitive to non-adherence (Kunz-Ebrecht et al., 2004; Pruessner et al., 1997; Hellhammer et al., 2007). Non-adherent subject may show a blunted CAR as well as reduced diurnal decline which may be misleading (Broderick et al., 2004; Kudielka et al., 2003). In addition there are a variety of psychosocial influences with different effects (Chida and Steptoe, 2009). The effect of non-adherence can be significant contributing as much as 30–40% of the variance (Almeida et al., 2009; Cohen et al., 2006). Other concerns include the accurate identification of true awakening time (Smyth et al., 2012). For example, do subjects consider awakening the time their eyes open at the sound of an alarm or define it otherwise? Novel approaches that facilitate reliable home-based collection are essential to move the field forward. We developed and validated the use of filter paper for collecting salivary cortisol from high risk infants hospitalized on a neonatal intensive care unit (Neu et al., 2007). Importantly this approach is not burdened by a requirement of sample refrigeration and can be stored dried at room temperature for an extended period without sample degradation. This opens up other situations in which this approach could be effectively applied (D’Anna-Hernandez et al., 2011; Kivlighan et al., 2008; Laudenslager et al., 2009). For adult studies we developed a novel packaging approach for collection of saliva samples in an organized, compact, and convenient manner that permitted recording of collection time directly on the collection booklet and not on separate log sheets. Improved methods for ensuring subject adherence are crucial for adult populations, particularly older groups (Kraemer et al., 2006). Accuracy of collection times indicated by subjects using our collection booklets has not been directly verified using electronic monitoring caps. The present study addressed several questions: (1) what is the agreement between the subject’s recordings of sampling times compared to the times recorded by an electronic monitoring cap? (2) are subjects adherent to specific post

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