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Sampling compliance for cortisol upon awakening in children and adolescents

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Summary Compliance with awakening salivary sampling is important for precise measurement of the diurnal cortisol profile. During childhood and adolescence, developmental factors influence sampling upon awakening ($awake_0$) due to school routine, sleep/wake patterns, and age related cortisol changes. In the present study, children and adolescents' sampling compliance of awakening cortisol was evaluated using accelerometry. Children and adolescents (N = 201; 45.3% female; 8—18 years; M_{age} = 12.68 years, SD = 2.03) participating in the Healthy Heart Project collected saliva samples, wore a tri-axle accelerometer, and completed demographic questionnaires. Intra-class correlations derived to examine $awake_0$ sampling compliance indicated children and adolescents were highly compliant (ICC = .98). In children, a delay in $awake_0$ sampling was associated with a steeper diurnal slope (β = —.23, p = .037) and greater $awake_0$ cortisol (β = .24, p = .024); this was not observed in adolescents. In summary, children and adolescents are compliant with awakening salivary sampling. Sampling delay, particularly in children, and time of awakening influenced measures of the diurnal cortisol profile. These findings inform future studies assessing the diurnal cortisol profile in children and adolescents.

1. Introduction

Compliance with salivary cortisol sampling is a requirement for the valid assessment of the diurnal cortisol profile. Cortisol levels change rapidly in the morning as part of the awakening response, when cortisol increases quickly, peaking approximately 30 min after wake-time (Young et al., 2004; Fries et al., 2009). To accurately capture the cortisol awakening response (CAR) individuals must be compliant with saliva sampling, which includes collecting a sample immediately upon waking ($awake_0$ sample). Compliance with this initial sample is also important for other measures of the diurnal cortisol profile (e.g., diurnal slope), as they too use the $awake_0$ sample in their calculation (Adam and Kumari, 2009; Rotenberg et al., 2012). To date, most research has exclusively focused on verifying self-reported sampling time with an objective measure of time, such as an electronic monitor that date- and time-stamps bottle opening and presumed time of saliva collection (e.g., MEMS Cap; Kudielka

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et al., 2003; Broderick et al., 2004). Although this research demonstrates that most adults report collecting their awake$_0$ sample within 10 min of the time reported by the electronic monitor (Broderick et al., 2004), this method does not verify sampling compliance against actual wake-time. Electronic monitors record when the sample was taken, but cannot indicate if there was a delay between wake-time and collection of the awake$_0$ sample (Clow et al., 2004; Dockray et al., 2008).

Emerging technology has allowed researchers to examine whether the awakening sample (awake$_0$) is taken at wake-time. Adults' compliance with sampling upon awakening has been investigated using accelerometry technology to detect physical movement and postural changes (Kupper et al., 2005; Dockray et al., 2008; DeSantis et al., 2010; Griefahn and Robens, 2011). Postural change from lying down (supine) to sitting up in bed or standing is deemed a valid proxy for waking in the sleep literature (Sadeh, 2011; Zeiders et al., 2011; Anders et al., 2012). In these adult accelerometry studies, only 15—19% of awake$_0$ samples were taken without delay, whereas 82—90% were taken within 15 min of wake-time (Dockray et al., 2008; DeSantis et al., 2010). Even this short delay can be problematic as later awake$_0$ samples result in blunted CAR and steeper diurnal slope (Kupper et al., 2005; Dockray et al., 2008; Okun et al., 2010; Griefahn and Robens, 2011). Further, Dockray and colleagues (2008) found that when there was a delay of more than 15 min between wake-time and collecting the awake$_0$ sample, estimates of CAR were lower than when there was delay of less than 15 min. These objective, accelerometry-based findings in adults suggest there may be an acceptable period in which the awake$_0$ sample can be collected (i.e., within 15 min) to yield reliable estimates of CAR.

There is a lack of research examining awake$_0$ sampling compliance in children and adolescents. Adult findings cannot be generalized to children and adolescents due to several developmental factors that influence the diurnal cortisol profile (Rotenberg et al., 2012). Developmental factors during childhood and adolescence influence awake$_0$ sampling (e.g., school routine, changes to sleep/wake pattern; Jessop and Turner-Cobb, 2008) as well as the cortisol response. First, when school is in-session, children and adolescents typically have a regimented morning routine (i.e., wake up, brush teeth, get dressed, eat breakfast) that ensures they catch the bus and arrive at school on time. For many, this routine occurs under pressured time constraints, which can limit their ability to accurately collect the awake$_0$ sample immediately upon waking. In contrast to adults, this morning routine is usually less internalized and self-governed. Second, night-time sleep duration decreases and morning drowsiness increases across childhood and adolescence (Carskadon, 1990; Sadeh et al., 2000; Fallone et al., 2002; Liu et al., 2005). Fewer changes in sleep habits are observed among adults. Feeling drowsy and less alert in the morning may contribute to children and adolescents forgetting to take the sample or less precision in the collection of their awake$_0$ sample. Relatedly, shorter sleep duration is associated with higher awake$_0$ cortisol levels (Rotenberg et al., 2012) and flatter diurnal slope (Zeiders et al., 2011). Further, adolescents commonly experience phase-shift delay, resulting in potentially greater morning fatigue and grogginess due to the propensity to sleep-in later, despite early school start times. Thus, adolescents may be less compliant with awake$_0$ sampling compared to younger children. Consistent with this idea, Jessop and Turner-Cobb (2008) suggest that children may collect the awake$_0$ sample more reliably than adolescents, due to varying degrees of parental supervision. Finally, the cortisol response differs across childhood and adolescence, as total cortisol concentrations increase steadily (Lupien et al., 2001; Walker et al., 2001; Tornhage, 2002; Gunnar et al., 2009). Pubertal maturation is also associated with a flatter diurnal slope (Rotenberg et al., 2012), increased cortisol (Kiess et al., 1995; Oskis et al., 2009), and reduced CAR (Adam, 2006). Given that these developmental factors may influence awake$_0$ sampling and the cortisol response, and in turn, that awake$_0$ sampling is important to accurately capture CAR and the diurnal cortisol profile, it is necessary to consider whether children and adolescents are compliant with awake$_0$ sampling.

Previous methodological studies have examined the stability of CAR and the diurnal profile in children and adolescents (see Oskis et al., 2009; Rotenberg et al., 2012). However, the methodological issue regarding awake$_0$ compliance has yet to be examined in childhood. The goal of the present study was to evaluate children and adolescents' compliance with collecting an awake$_0$ sample validated against accelerometry, as an objective measure of wake-time. Based on previous findings, it was hypothesized that children and adolescents would be highly compliant with collecting an awake$_0$ sample, with children more compliant than adolescents. The effect of a delay between wake-time and collecting the awake$_0$ sample on measures of the diurnal cortisol profile was also examined. It was hypothesized that a greater delay would yield lower estimates of the cortisol awakening response and diurnal cortisol profile.

2. Method

2.1. Participants

Children and adolescents aged 8—18 years were recruited to take part in the larger Healthy Heart Project, a longitudinal study examining early cardiovascular risk factors, at Concordia University, Montreal, QC. Flyers, postcards, and bookmarks were distributed throughout the community and in schools approved by the Montreal English School Board. Children with serious psychopathology or prescription medication use were excluded. During the study, participants were asked to refrain from using over-the-counter medications and caffeine. Parental and adolescent informed consent and child assent were obtained. This study was approved by the Concordia University Ethics Review Committee (UH2005-077).

2.2. Measures

2.2.1. Wake-time

Children and adolescents wore an undergarment vest that contained an embedded tri-axle accelerometer for 24 h for the Healthy Heart Project protocol. The accelerometer was fitted securely around the abdomen, and differentiated supine from upright posture. Accelerometry data was processed using VivoLogic Version 3.2 (VivoMetrics Inc.) and